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Processing of Plasmid DNA During Bacterial Conjugation

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INTRODUCTION

Conjugation is the highly specific process whereby DNA is transferred from donor to recipient bacteria by a mechanism involving cell-to-cell contact. This process is usually encoded by conjugative plasmids, which have been isolated from a diverse range of gram-negative bacteria and include members of more than 20 incompatibility groups (listed in Appendix B of reference 32; 24, 53). All of these plasmids encode conjugative pili necessary for establishing cell-to-cell contact, and their molecular mechanisms of conjugation may be similar. However, it is likely that many genetically distinct conjugation systems exist, since plasmids from one incompatibility group or family do not complement transfer-deficient mutants of another. Also, plasmids belonging to different incompatibility groups generally show little DNA homology (see reference 53), and conjugative ability requires an extensive region of plasmid DNA (comprising about one-third of the genome or 33 kilobases [kb] in the case of F; Fig. 1). Naturally occurring plasmids belonging to the same incompatibility group usually share extensive DNA homology and encode related pili and conjugation systems (25, 53), although this relationship is evolutionary and not obligatory. The transfer mechanisms of conjugative plasmids in gram-positive bacteria are likely to be biochemically as well as genetically distinct from those in gram-negative species since these plasmids do not encode pili for recognition of potential recipient cells (see reference 37 for review).

This review will concentrate on the central aspect of conjugation: the processing of plasmid DNA during its conjugative transfer. This processing includes nicking and initiation of DNA transfer at the origin of transfer site, separation of the two plasmid DNA strands, strand transfer, conjugative DNA synthesis in both donor and recipient cells, and recircularization of the conjugatively replicated plasmid molecules. Discussion will necessarily be limited to plasmids that have been studied in *Escherichia coli*, those with the best understood conjugation systems being IncF plasmids including, in particular, F itself (reviewed most recently by Willetts and Skurray [186]). We shall examine carefully the extent to which information on F transfer is known to hold for the conjugation systems of plasmids in other incompatibility groups, since F is often taken as a model. Also, we shall review the molecular mechanisms by which certain conjugative plasmids mobilize small (<10 kb) naturally occurring nonconjugative plasmids such as ColE1, the related plasmid CloDF13, and the broad-host-range IncQ plasmid RSF1010.

This review will not encompass the cellular interactions that occur during conjugation, including the nature and diversity of conjugative pili, and the formation and dissociation of mating pairs or aggregates. These aspects have recently been reviewed by Bradley (25), Manning and Achtman (116), and Willetts and Skurray (186).

TRANSFER OF DNA

Single-Strand Transfer

The fate of the two component DNA strands of a plasmid during conjugation was first analyzed with F, and this plasmid remains the best documented in this regard. The finding that minicell recipients accumulated predominantly single-stranded DNA during F-mediated conjugation suggested that only one strand of the plasmid is transferred (42, 43). Analysis of which strand of λ prophage was transmitted from Hfr or F-prime donor strains showed that a unique strand is indeed transferred, with the 5′ terminus leading (92, 130, 141). It was subsequently found that this strand of F

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is the denser of the two in polyuridylate-polyguanylate [poly(U,G)]-CsCl gradients and that the lighter is retained in the donor cell (165, 166), thus ruling out the possibility that a strand is degraded in the transfer process. By selectively labeling DNA in either the donor or the recipient cell, it was demonstrated that DNA is synthesized on the transferred F strand in the recipient (130, 165) and that the strand retained in the donor serves as template for synthesis of DNA to replace that transferred (165, 166). After mating, such conjugatively replicated DNA was recoverable from both donor and recipient cells in the form of covalently closed circular molecules. IncFII plasmid R538-1 also transfers a specific strand which is likewise the more dense in a poly(U,G)-CsCl gradient (164). Given the similarity of the conjugation systems of IncF plasmids, it is a reasonable assumption that plasmids of this group are all transferred by the same overall mechanism as F itself.

The IncIα plasmid R64*drd-11* also transfers the strand that preferentially binds poly(U,G) (166), and other plasmids of this group such as ColIb-P9 and R144 are presumably similar in this regard. Although the gradient of recombinants generated in matings with R144-mediated Hfr donor strains indicates unidirectional transfer of DNA (54), the chemical polarity of the transferred IncIα plasmid strand has yet to be established. The molecular nature of the DNA transmitted by other groups of conjugative plasmids has not been studied and caution should be exercised in extrapolating to these the information gained for F. For only one, the IncP plasmid RP4, has it even been shown that transfer is unidirectional (6, 81).

The same paucity of information obtains for the numerous small nonconjugative plasmids that are mobilizable. In no case has it been demonstrated that a unique strand is transferred or that DNA is transferred with a leading 5′ terminus. However, if the "relaxation complexes" (see section, "Nicking at *oriT*") formed by many nonconjugative plasmids can be taken as an accurate in vitro representation of events occurring during conjugation in vivo, transfer of a unique plasmid strand would be expected since a unique strand is nicked.

Route Through the Cell Envelope

The pilus is required at least for the cell-to-cell interactions that precede DNA transfer, although it is not known whether it forms the bridge through which the DNA passes from the donor to recipient cell. Representative conjugative plasmids belonging to all incompatibility groups tested in *E. coli* K-12 and in *Pseudomonas* spp. are known to specify pili (24, 25), and a role in conjugation has been demonstrated for F (128), I (88), W (23), and N and P (26) pili. The F pilus (see references 116 and 182 for recent reviews) is the best studied; it forms a specific contact with the potential recipient cell and its retraction brings the cells into wall-to-wall contact. The pairs or aggregates thus formed are initially sensitive to shear forces but are then stabilized by an unknown process requiring the products of *traG* and *traN* (117). There is indirect evidence that I pili also retract; their production by *E. coli* cells is stimulated by I-pilus antibody, and this was interpreted as reflecting inhibition of pilus retraction (110).

Presumably a channel is formed between the juxtaposed donor and recipient cell envelopes to allow DNA transfer. This channel might be equivalent to a "stub" of the pilus, which has an axial hole just large enough to accommodate single-stranded DNA (71). In accordance with this, sodium dodecyl sulfate at a concentration that depolymerizes F pili did not prevent DNA transfer once mating aggregates had been formed (4). There is no conclusive evidence to resolve whether or not plasmid DNA can utilize the extended pilus as a bridge for entry into the recipient cell, despite efforts in this direction (135).

An alternative model is that donor and recipient cell envelopes fuse locally at the point of contact and that a transmembrane "pore" is formed through which DNA is transferred. Evidence favoring this concept is provided by the report that phage λ receptor sites are exchanged bidirectionally between Hfr and F^- cells as a consequence of conjugation (R. Goldschmidt and R. Curtiss, unpublished data in reference 48). Such a union might also allow efficient transfer of specific plasmid proteins required for conjugative DNA metabolism in the recipient cell, since F *tra* gene products are largely membrane located (98) as are, at least in the case of R64*drd-11* (55, 67) and R1*drd-19* (64), conjugatively replicating plasmid DNA molecules in donor and recipient cells.

The product of the F *traD* gene is located in both the inner and outer cell membranes (3), and by analogy with its role in penetration into the cell of the RNA of some pilus-specific phages (136), it may also be required for transport of plasmid DNA through the cell envelope. Alternatively, it might have a role in strand separation (99). The *traD* protein has been purified, but no DNA binding or ATPase activity has been discovered in preliminary experiments (D. Panicker and E. Minkley, personal communication). It is of interest that the *traD* product is essential for the mobilization of ColE1 (7, 182) but not CloDF13 (161, 182).

ORIGINS OF TRANSFER

The *oriT* Site

Transfer of plasmid DNA is initiated at a specific site on the plasmid molecule denoted the origin of transfer or *oriT*. Such a site on F was first recognized in deletion mapping experiments and was located at one end of the transfer region (85, 86, 180). Figure 1 shows a physical map of the F transfer region, including *oriT*; this site is asymmetric and oriented such that the transfer region is transferred last. Similar experiments indicate that IncP (RK2 and RP4) and IncN (R46 and N3) plasmids also possess an *oriT* region located in an analogous position with respect to their conjugation genes (6, 30, 83; A. Brown and N. Willetts, unpublished data).

Since *oriT* is a specific site, and plasmids containing such a sequence can be selected via their efficient transfer from bacteria carrying a compatible conjugative plasmid to provide the necessary *trans*-acting functions, cloning techniques are ideal for the isolation and analysis of DNA fragments carrying an *oriT* site. For example, the F *oriT* site was initially cloned on an 8.3-kb *Eco*RI fragment (5) and then on a 1.1-kb *Bgl*II fragment (154), a 540-base pair (bp) *Bgl*II-*Sal*I fragment (97), and a ca. 385-bp *Bgl*II-*Hae*II fragment (62). It should be noted that the latter lacks the contiguous 156-bp *Hae*II-*Sal*II fragment previously thought to contain *oriT* (155).

Short DNA fragments containing the *oriT* site of other conjugative plasmids have also been cloned. These include R1 and R100 (IncFII; see Table 1), ColIb-P9 (IncIα; C. Wymbs and B. Wilkins, unpublished data), RK2 and RP1 (IncP; 83, 84, 173; K. Derbyshire and N. Willetts, unpublished data), and R46 (IncN; A. Brown, G. Coupland, and N. Willetts, unpublished data). Examples of nonconjugative plasmids whose *oriT* regions have been cloned are ColE1 (172; Fig. 2), its close relative pMB1 (46, 68), and RSF1010

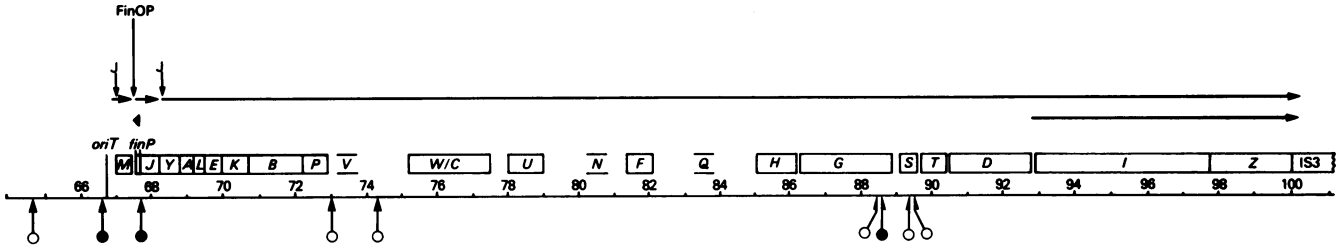


FIG. 1. Physical and genetic map of the transfer region of plasmid F. The numbers show kilobase coordinates and the horizontal lines above the genes represent transcripts. The direction of DNA transfer from *oriT* is such that the transfer region is transferred last. The *traM* and *traJ* promoter regions have been sequenced (156) and the *traY-Z* operon has been shown to have its own separate promoter (P. Mullineaux and N. Willetts, unpublished data). *finP* may be transcribed from the DNA strand opposite the long leader sequence of the *traJ* mRNA (N. Willetts, J. Maule, and R. Thompson, unpublished data). The vertical arrows with open and closed circles indicate the positions of *Eco*RI and *Bgl*II cleavage sites, respectively. Transcription from the promoters for *traM* and for the *traY-Z* operon is dependent on the product of *traJ*, which is in turn negatively regulated by the FinOP repressor (68, 73, 181). The *traI* and *traZ* genes are transcribed constitutively from a second promoter at about 18% of the level from the *traJ*-induced *traY-Z* operon promoter (Mullineaux and Willetts, unpublished data). Roles attributed to the genes are: regulation, *finP* and *traJ*; pilus formation, *traA*, *-L*, *-E*, *-K*, *-B*, *-V*, *-W/C*, *-U*, *-F*, *-Q*, *-H*, and *-G*; stabilization of mating pairs, *traN* and *-G*; conjugative DNA metabolism, *traM*, *-Y*, *-D*, *-I*, and *-Z*; surface exclusion, *traS* and *-T*. Further details are given in Willetts and Skurray (186).

(IncQ; Derbyshire and Willetts, unpublished data). The above examples have confirmed that all of these plasmids do indeed carry a relatively short *oriT* site required in *cis* for conjugative transfer.

Cloning followed by sequencing of *oriT* DNA fragments has been used to determine the nucleotide sequences of the *oriT* regions of several plasmids including F (R. Thompson, L. Taylor, K. Kelly, R. Everett, and N. Willetts, submitted for publication), RK2 (84), ColE1 (13), pMB1 (on pBR322; 145, 153), pSC101 (126), and RSF1010 (Derbyshire and Willetts, unpublished data). Some of these sequences are shown in Fig. 3, together with regions of dyad symmetry which might be important for their *oriT* function.

Nicking at *oriT*

There is indirect evidence from two types of experimental systems suggesting that a nick is introduced at the *oriT* site in a specific plasmid strand. First, mild lysis of host cells has allowed the isolation of a complex consisting of superhelical plasmid DNA and proteins that can introduce a nick into one of the plasmid strands when treated with such agents as ionic detergents, proteases, or ethidium bromide. The best studied relaxation complex is that of ColE1 (38, 39), for which it has been shown that the DNA is associated with three distinct proteins of 60,000, 16,000, and 11,000 daltons (112). Although at least one of these proteins (the 16,000-dalton polypeptide) is thought to be encoded by a *mob* gene of ColE1 itself (44, 57, 95), the provenance of the others, whether from plasmid or host genes, is unknown.

Upon relaxation of the ColE1 complex, the 60,000-dalton protein becomes covalently linked to the 5′ terminus of the nick, whereas the two smaller proteins dissociate (15, 82, 112). The nick is located in the heavier (H) of the two strands in a poly (U,G)-CsCl gradient at a specific location (designated *nic*; 172) about 270 bp from *oriV*, the origin of vegetative replication (13, 14, 111, 151, 158; Fig. 2). The relaxation complex is implicated in conjugation since ColE1 mutants deficient in forming the complex are mobilized inefficiently (45, 57, 94). Furthermore, the site at which the mobility proteins act has been located at or close to the *nic* site, using *cis*-acting deletion mutations that prevent mobilization (172). Hence, *nic* is presumably the same as *oriT* and nicking at this site, together with linkage of the resulting 5′ terminus to the 60,000-dalton protein, may be a prelude to DNA transfer.

Similar strand- or site-specific nicking, or both, of relaxation complexes has been reported for other nonconjugative plasmids, including ColE2 (14, 111), pSC101 (127), and RSF1010 (127), and for the conjugative plasmids F (100; although a subsequent study [97] failed to reveal *oriT*-specific nicking), R12 (IncFII; 124), RK2 (IncP; 83), and R6K (IncX; 106, 127). Although two IncIα plasmids, ColIb-P9 and R64, can also be isolated as relaxable complexes of supercoiled DNA and protein (40, 107), they need further examination since neither a strand-specific nor a site-specific nick has been demonstrated and a nonspecific endonuclease may have been responsible for the nicking.

A second system that allows recognition and characterization of a nick at the *oriT* site has been developed for F by Everett and Willetts (61) and may also be applicable to other plasmids. It is based upon the observation that when a λ *oriT*$^+$ transducing phage, constructed in vivo or by in vitro cloning techniques, is grown in F *tra*$^+$ cells, about 10% of the phage genomes in the resultant lysate contain an interruption in one DNA strand. This "nicking" appears to be a conjugation-dependent phenomenon since it occurred only when the phage was *oriT*$^+$ and the cells carried F *tra*$^+$, and it took place in the strand that is transferred during conjugation (61, 62). The 3′ terminus at the interruption contained a hydroxyl group since it was susceptible to exonuclease III and primed DNA synthesis by DNA polymerase I. However, the nicked DNA could not be religated with T4 ligase, although it was insensitive to endonuclease S1, indicating that either the 5′ terminus at the nick is modified in some unknown way or the strand interruption actually consists of the deletion or addition of a small number of bases (61; Thompson et al., submitted).

The nick sites have been located on the DNA sequences of ColE1 (13), pSC101 (126), and F (62; Thompson et al., submitted), using nicked relaxation complex DNA or λ *oriT*$^+$ genomes as the starting material. The nick sites are shown in Fig. 3. In ColE1 and pSC101, the nick is near one end of a region of imperfect twofold rotational symmetry, and it is presumably at a similar location in the related imperfect symmetry regions of pMB1 and CloDF13. No such regions exist in F, RK2, or RSF1010. In F, there were apparently three or more nick sites, used with different frequencies; it remains to be determined whether these

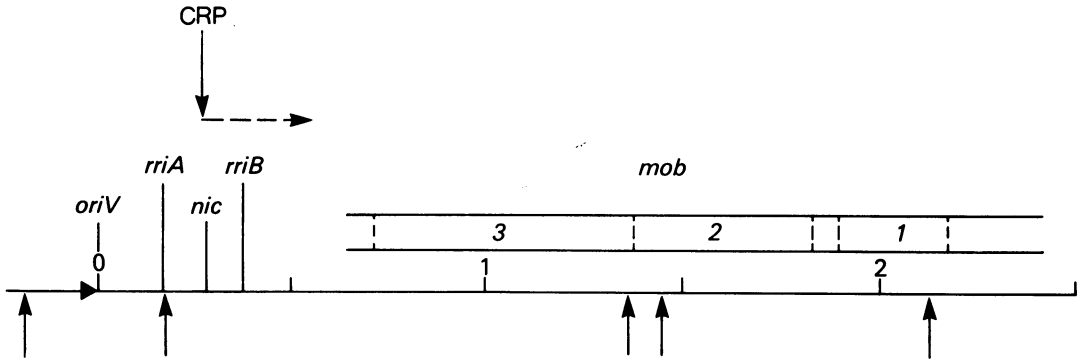


FIG. 2. Map of the conjugative mobility region of plasmid ColE1. The map is based on that of Dougan et al. (56) and a nucleotide sequence of part of this region (132). Kilobase coordinates extend from the origin of replication (*oriV*; 158), the arrowhead indicating the rightward direction of fork movement. Verticle arrows indicate the position of *Hae*II cleavage sites, *nic* is the putative origin of DNA transfer (see text), the pair of horizontal lines indicates the extent of the mobility region mapped using transposon insertion mutants (56), and the vertical dashed lines show the approximate limits of segments defining three complementation groups, *mob-1*, *-2*, and *-3* (96). *rriA* and *rriB* are presumptive assembly sites for primosomes that will generate primers to initiate leftward and rightward DNA synthesis, respectively (16, 125). The mobility region is thought to be transcribed from left to right (58); the horizontal broken line indicates the direction of transcription from a cyclic AMP receptor protein-dependent promoter (CRP; 137) identified immediately to the left of *nic* in the homologous region of pBR322 (derived from the ColE1-like plasmid pMB1).

multiple sites have biological significance or whether they result from the λ *oriT* methodology. For example, nick translation and endonucleolytic cleavage of the displaced strand might be necessary to remove a protein covalently bound to the true 5′ *oriT* terminus and thus to allow packaging of the λ *oriT* DNA. Centered 126 bp to the right of the strongest λ *oriT* nick site is a striking region of dyad symmetry that is related in sequence to similarly placed inverted repeat sequences in ColE1 and CloDF13 but not RK2 or RSF1010; this region may be the recognition site of a host- or plasmid-encoded protein necessary for mobilization by the F conjugation system. Between the *oriT* nick sites and this region of dyad symmetry is an adenine-thymine-rich region (nucleotides 148 to 234; ca. 80% adenine-thymine) which could aid initial strand separation: an adenine-thymine-rich region is common to the vegetative replication origins of several plasmids and bacteriophages.

Use of bacteria containing F *lac tra* point and deletion mutants in conjunction with λ *oriT*$^+$ phages showed that in vivo nicking at *oriT* requires the products of *traY*, *traZ*, and *traJ* (61). The *traJ* protein plays a regulatory role in transcription of the *tra* operon and its role in the nicking process may therefore be indirect. Interestingly, *traY* and *traZ* are 30 kb apart, at the extremes of the large *traY-traZ* operon (Fig. 1). The *traY* product has been located in the cell envelope (116) and the *traZ* product (protein "2b") has been located in the cytoplasm (3); this may place a complex of the two, together with *oriT*, strategically at the boundary of the cytoplasm and cell membrane. Regrettably, no *traY* or *traZ* point mutants of F have been isolated so far.

The recognition site of the F *traYZ* endonuclease activity has been partially defined by determining the sequences of several *oriT* mutants. These were isolated by taking advantage of the pronounced instability, in the presence of F *lac*, of a pBR322 derivative with *oriT* cloned in one particular orientation; stable mutants proved to carry *oriT* mutations (62). Eleven independent mutations were found to be C → T transitions at either position 141 (two mutations) or 146 (nine mutations) which lie 14 or 19 bp to the 5′ side of the major λ *oriT* nick (Thompson et al., submitted) (Fig. 3). The mutation at position 141 was shown to prevent nicking when the mutant plasmid sequence was cloned into a λ vector and the resultant λ *oriT*$^-$ phage was grown in the presence of F *tra*$^+$ (62). This base pair is located in one, but not the other, of two alternative inverted repeat sequences (Fig. 3), but it is not known whether these structures play a role in the recognition of *oriT* by the *traYZ* endonuclease. If the multiple λ *oriT* nicks are derived by nick translation from the true *oriT* nick site as suggested above, the *oriT* mutation at position 141 could be very close to this site. More extensive sequencing studies of *oriT* mutants, together with the establishment of an in vitro system for nicking at *oriT*, are required to complete our understanding of this first stage in conjugative DNA metabolism.

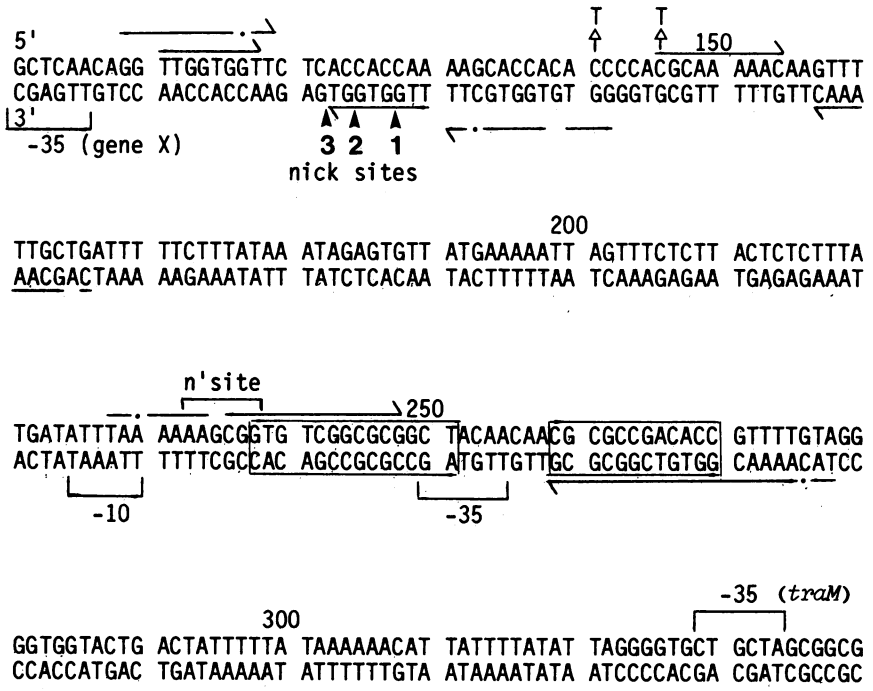
Initiation of DNA Transfer

DNA transfer and conjugative DNA synthesis are initiated in response to an as yet unidentified signal generated by mating-pair formation. This signal does not appear to be necessary to trigger nicking at *oriT*, since λ *oriT*$^+$ phages were nicked when grown in cells carrying F mutants unable to synthesize a pilus. Nicking and religation at *oriT* may therefore occur continuously in donor cells, giving an equilibrium between the covalently closed and *oriT*-nicked forms of the plasmid (61).

Initiation does not result from de novo synthesis of *tra* gene products since these are all expressed constitutively under positive control by *traJ* (181). Furthermore, pretreatment of donor cells with rifampin did not prevent transfer of F (99, 178) or mobilization of ColE1 (N. Willetts and J. Maule, unpublished data). These findings also make it unlikely that *oriT* is activated by transcription over this region from the nearby promoters recognized in the sequences of F and ColE1 (Fig. 3; see below), as suggested elsewhere for ColE1 transfer (69). Hence, mating-pair formation may cause an allosteric change in structure of a key protein or alteration of its intracellular location, with consequent activation of *oriT*.

One candidate for this protein is the *traM* product, which although not required for pilus synthesis, formation or stabilization of mating pairs, or nicking at *oriT*, is essential for DNA transfer and conjugative DNA synthesis in the donor cell (99). Consistent with this, there are three alleles of *traM* among IncF plasmids (Table 1 in reference 186) corre-

a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CGAGTTGTCC AACCACCAAG AGTGGTGGTT TTCGTGGTGT GGGGTGCGTT TTTGTTCAAA
3'
-35 (gene X)
3 2 1
nick sites
150
200
TTGCTGATTT TTCTTTATAA ATAGAGTGTT ATGAAAAATT AGTTTCTCTT ACTCTCTTTA
AACGACTAAA AAGAAATATT TATCTCACAA TACTTTTTAA TCAAAGAGAA TGAGAGAAAT
n'site
250
TGATATTTAA AAAAGCGGTG TCGGCGCGGC TACAACAACG CGCCGACACC GTTTTGTAGG
ACTATAAATT TTTTCGCCAC AGCCGCGCCG ATGTTGTTGC GCGGCTGTGG CAAAACATCC
-10
-35
-35 (traM)
300
GGTGGTACTG ACTATTTTTA TAAAAAACAT TATTTTATAT TAGGGGTGCT GCTAGCGGCG
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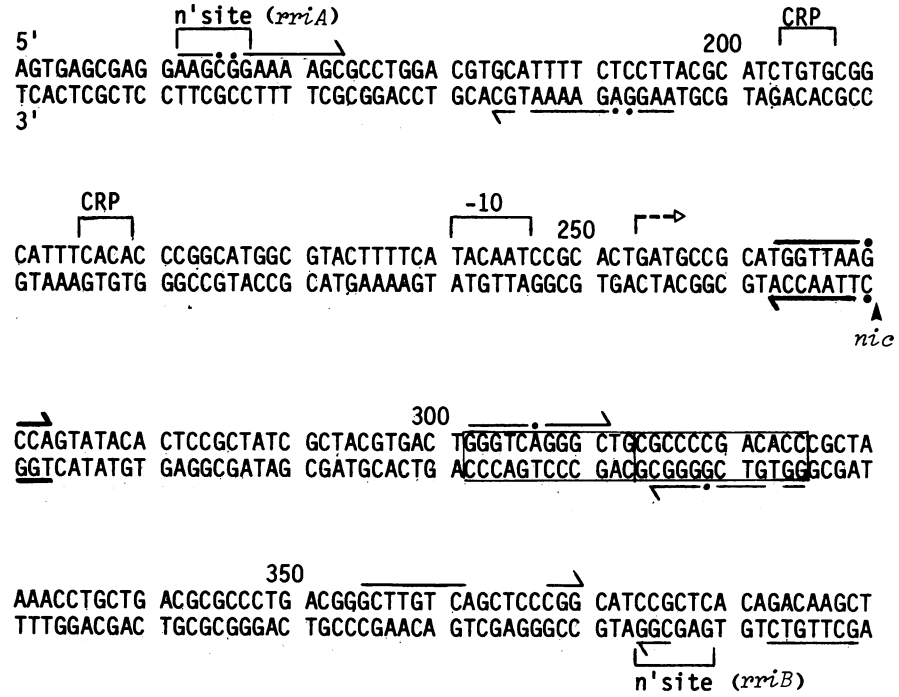
b


n'site (rriA)
CRP
5'
200
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TCACTCGCTC CTTCGCCTTT TCGCGGACCT GCACGTAAAA GAGGAATGCG TAGACACGCC
3'
CRP
-10
250
CATTTCACAC CCGGCATGGC GTACTTTTCA TACAATCCGC ACTGATGCCG CATGGTTAAG
GTAAAGTGTG GGCCGTACCG CATGAAAAGT ATGTTAGGCG TGACTACGGC GTACCAATTC
nic
300
CCAGTATACA CTCCGCTATC GCTACGTGAC TGGGTCAGGG CTGCGCCCCG ACACCCGCTA
GGTCATATGT GAGGCGATAG CGATGCACTG ACCCAGTCCC GACGCGGGGC TGTGGGCGAT
350
AAACCTGCTG ACGCGCCCTG ACGGGCTTGT CAGCTCCCGG CATCCGCTCA CAGACAAGCT
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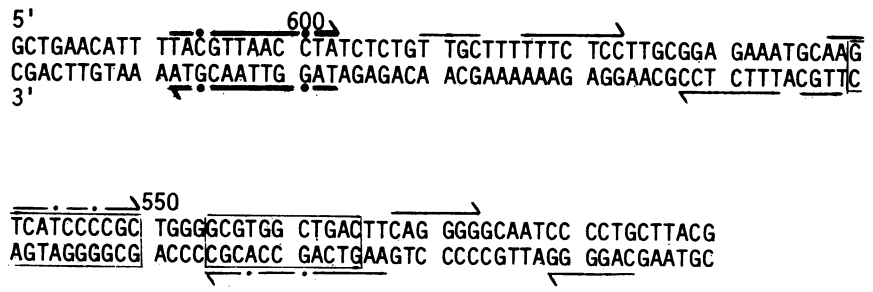
n'site (rriB)

c


5'
600
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CGACTTGTAA AATGCAATTG GATAGAGACA ACGAAAAAAG AGGAACGCCT CTTTACGTTC
3'
550
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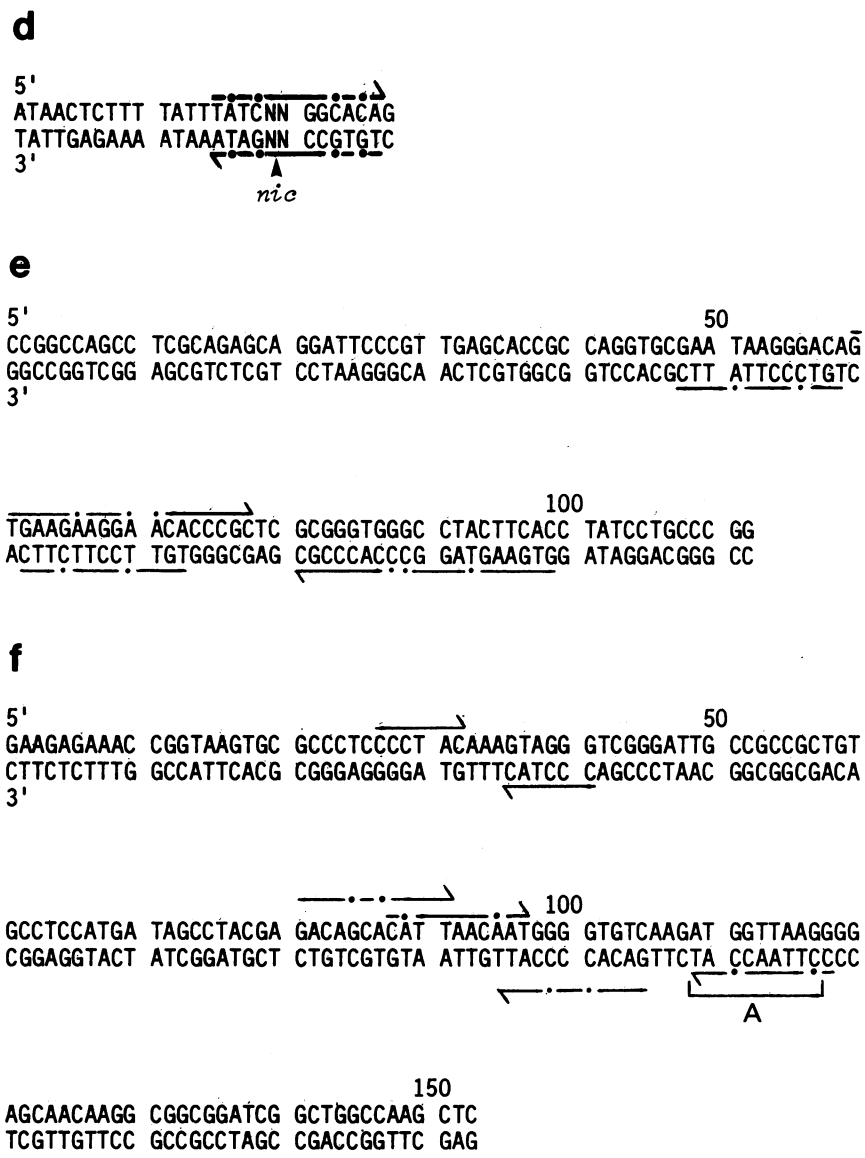

FIG. 3. Sequences of *oriT* regions. *oriT* region of F, located in the intercistronic region between gene "X" (an unassigned open reading frame) and *traM*. Numbering is from the center of the *Bgl*II site located to the left of *oriT*. Putative promoter (-35, -10) sequences and a possible protein n′ recognition site are marked, as are inverted repeat sequences, the three major *oriT* nick sites (1, 2, 3 in the lower strand), and the C→T transitions at positions 141 and 146 that give *oriT* mutations. Sequences similar to those in boxes also form inverted repeats in ColE1 and CloDF13. The data for *traM* are taken from Thompson and Taylor (156) and those for *oriT* are from Thompson et al. (submitted). (b) *oriT* region of ColE1. The nucleotide sequence was taken from Oka et al. (132). Numbering is from the origin of replication (158). The upper and lower lines are the L and H strands, respectively. The most likely position of the relaxation nick site (*nic*), as proposed by Bastia (13), is shown (▲), although its precise location may be one or two nucleotides away from this. The imperfect twofold rotational symmetry of the region including *nic* and prominent inverted repeats are shown (13, 16, 118). The boxed sequences are related (19 of 24 bases) to part of the large inverted repeat in F between nucleotides 228 and 278. The pBR322 (derived from pMB1) sequence in the *nic* region (153) is closely similar to that of ColE1, the region of twofold rotational symmetry being between coordinates 2,251 and 2,261. Hexanucleotides that may be recognized by protein n′ and contribute to the *rriA* and *rriB* primosome assembly sites (16, 118, 125) are marked. The -10 sequence of the predicted cyclic AMP receptor protein-dependent promoter, the $TGTGN_8CACA$ symmetry in its cyclic AMP receptor protein binding site, and the initiation site of rightwards transcription (- - - ▷) are shown (137). (c) *oriT* region of CloDF13 (A. Snijders, A. van Putten, E. Veltkamp, and H. J. Nijkamp, personal communication.) Position 604 is the *Hpa*I coordinate reference point. The origin of transfer is probably located in the lower strand as drawn, within the region of imperfect twofold rotational symmetry (609 to 598) since this is similar to the *oriT* regions of ColE1 and pMB1, and a Tn*901* insertion within it (161) caused a dominant *oriT* mutation. The boxed inverted repeat sequences (561 to 536) are related to the similarly placed inverted repeats in ColE1 (12 of 20 bases) and F (13 of 20 bases). *oriV* (149) and a promoter sequence comparable to the cyclic AMP receptor protein-dependent promoter of pBR322 (137) are located ca. 285 and 100 bp, respectively, to the left of *oriT* as drawn. (d) *nic* region of pSC101, taken from Nordheim (126). Unknown bases are designated N, and the *nic* site (▲) and a possible region of imperfect twofold rotational symmetry are marked. (e) *oriT* region of RK2 on a 112-bp *Hpa*II fragment (84). A large inverted repeat is marked with arrowheads, and a direct repeat is underlined. (f) *oriT* region of RSF1010 on a 153-bp *Taq*I fragment (Derbyshire and Willetts, unpublished data). Three sets of inverted repeats are indicated. The sequence of nine nucleotides marked A is the same as that immediately to the 5′ side of the relaxation nick site in ColE1, and the origin of DNA transfer may be located within or near this region.

sponding to the three different *oriT* sequences described in the next section, and mobilization of ColE1, which has yet another *oriT* sequence, does not require the F *traM* product (161, 182). Moreover, the *traM* protein is appropriately located in the inner membrane of the cell (3, 156), and preliminary experiments indicate that it binds specifically to the 1.1-kb *BglII* fragment of F that includes *oriT* (D. Musgrave and M. Achtman, personal communication).

oriT Specificity

oriT sequences differ between plasmids and are highly specific. Even among IncF plasmids, which encode closely related pili and have genetically similar transfer regions, three different *oriT* sequences have been recognized. Table 1 shows that derivatives of the non-mobilizable vector plasmid pED825 carrying the cloned *oriT* regions of F *lac*, R1*drd-19*, or R100*drd-1* were only mobilized efficiently by the parental conjugative plasmid. As a corollary, the alleles of *traY* and *traZ* (and of any other genes with products acting at *oriT*) should also vary between the different IncF plasmids, and genetic data indicate that this is indeed the case (see Table 1 in reference 186 and Table 2 in reference 183).

The *oriT* sequences of nonconjugative plasmids also differ from each other. This has been directly demonstrated by sequencing studies of the *oriT* regions of ColE1, RSF1010, and pSC101 (Fig. 3) and indirectly by genetic studies. For example, since the *mob* gene products probably interact with *oriT*, the failure of ColE2 to complement ColE1*mob* mutants (171, 172) suggests that the *oriT* sequences of ColE1 and ColE2 differ, even though both are mobilized by the same IncI conjugative plasmids. Nonconjugative plasmid *oriT* sequences also differ from those of conjugative plasmids able to mobilize them, as exemplified by the dissimilarity of the *oriT* sequences of F and ColE1 or CloDF13 and of RK2 and RSF1010 (Fig. 3). In accordance with these differences, the *traYZ* endonuclease of F is not required for transfer of either ColE1 (61, 185) or CloDF13 (182). Presumably the mobilization genes of ColE1 (or CloDF13) provide the necessary nicking function, although the precise role of these gene products, and even their number and sizes, have not yet been fully established (44, 57, 95, 96; M. Saul and D. Sherratt, personal communication).

Further consideration of the mobilization of nonconjugative plasmids reveals that, in addition to the specificity of interaction between their *oriT* sequences and their *mob* gene products, a second specific interaction must be invoked to explain why they differ in their ability to be mobilized by various conjugative plasmids. For example, ColE1 is mobilized efficiently by IncF, IncI, and IncP plasmids but weakly by IncW plasmids (138, 170). ColE2 is mobilized at a much higher frequency by IncI than by IncF plasmids (89), whereas RSF1010 is mobilized efficiently by IncP plasmids but only poorly by IncI and not at all by IncF plasmids (184). Further examples are given elsewhere (183). This type of specificity is also apparent among conjugative plasmids, since, for example, pilus-deficient F *tra* mutants, still expressing the gene products that interact with *oriT*, were not transferred by an IncIα plasmid (179). It is clearly necessary that the conjugative plasmid's system for physical transfer of DNA should recognize the nonconjugative plasmid's *oriT* mobilization system, or possibly a second DNA sequence other than *oriT* (183).

TABLE 1. Mobilization of *oriT* recombinant plasmids

Conjugative plasmid	Mobilization[a]		
	pED822 (*oriT*-F)	pED221 (*oriT*-R1)	pED222 (*oriT*-R100)
F *lac*	140	6×10^{-4}	2×10^{-3}
pED219[b]	2×10^{-3}	112	4×10^{-3}
R100*drd-1*	0.3	7×10^{-3}	205

[a] Expressed as a percentage of the transfer of the conjugative plasmid. pED221, pED222, and pED822 are derivatives of the 2.25-kb vector plasmid pED825 carrying the *oriT* sites of the conjugative plasmids on 0.4- to 1.6-kb fragments (62; N. Willetts, R. Everett, W. Smith, and J. Maule, unpublished data).

[b] pED219 is a derivative of R1*drd-19* determining only kanamycin resistance.

STRAND SEPARATION

After nicking at *oriT* of the strand destined for transfer, the two DNA strands of a plasmid must be unwound to allow transmission of a single strand to the recipient cell. It has recently been demonstrated that DNA helicase I, a DNA unwinding enzyme isolated from F-containing cells, is the product of the F *traI* gene (2a). This fibrous protein, which unlike most *tra* gene products is located in the cytoplasm (3), has a molecular weight of about 180,000 (185) and it possesses a DNA-dependent ATPase activity (1). In vitro studies with phage DNA substrates (2, 105) have revealed that unwinding may first require the binding of 70 to 80 molecules of the protein to a single-stranded region of DNA of about 200 nucleotides. The resulting multimer may then migrate as a stable complex along this strand, using the energy of ATP hydrolysis for DNA unwinding. Migration proceeds in the 5′ to 3′ direction, and the estimated rate of unwinding by DNA helicase I is about 1,200 bp/s (105).

Since *traI* mutants of F can promote formation of stable mating pairs but are defective in DNA transfer and conjugative DNA synthesis in the donor (99), DNA helicase I is presumably required for unwinding the DNA strands of F as an essential preliminary to DNA transfer. In this role, the enzyme might bind near the 5′ terminus of the nicked strand and then processively unwind the helix, thus displacing the bound strand into the recipient cell (Fig. 4). The rate of unwinding stated above is similar to the rate of F-determined chromosomal DNA transfer by Hfr strains. The F *traI* product is not interchangeable with that of the closely related IncFII plasmid R100*drd-1* (185), indicating that its interaction with F DNA or other F *tra* products is specific. In vitro studies of DNA helicase I, using a DNA substrate that includes the F *oriT* sequence rather than bacteriophage DNA, might therefore be instructive. The F *traI* product is not required for mobilization of ColE1 (7, 182, 185) or CloDF13 (161, 182), so presumably these must either contribute their own unwinding protein or utilize a host-encoded product.

DNA gyrase (*Eco* DNA topoisomerase II), an *E. coli* enzyme that converts relaxed closed circular duplex DNA to a negatively supercoiled form (see reference 77), may also be involved in conjugative DNA transfer. This is suggested by genetic and molecular studies with nalidixic acid, which is an inhibitor of gyrase (150) and acts in sensitive donor cells to inhibit initiation and continuation of transfer and conjugative synthesis of F plasmids (12, 42; R. Everett and N. Willetts, unpublished data), R64*drd-11* (67), and chromosomal DNA of Hfr strains (17, 28, 49, 70, 87, 91). By analogy with the possible role of gyrase in replication of closed circular duplex DNA (77), the enzyme may be required in conjugation to promote protein-DNA interactions necessary for triggering DNA transfer and, if axial rotation of the opened

strand is restrained, to facilitate DNA unwinding. However, it is premature to conclude that gyrase-induced DNA supercoiling is essential for the transfer process since there is evidence from other systems that nalidixic acid may cause gyrase-DNA complexes that block DNA metabolism and break DNA independently of its effects on supercoiling (see reference 77 for review). Thus, the role of gyrase in conjugative DNA transfer needs to be evaluated by other approaches involving, for example, temperature-sensitive *gyr* mutants (104, 133).

CONJUGATIVE DNA SYNTHESIS

Transfer of a single strand of plasmid DNA is normally associated with synthesis of a replacement strand in the donor cell (termed DCDS) and of a complementary strand in the recipient (RCDS). Most of the information on this DNA synthesis stems from studies of F and of two transfer-derepressed IncIα plasmids, ColIb-P9*drd-1* and R64*drd-11*, all of which have the necessary attribute of promoting efficient conjugation in liquid culture. Conjugative DNA synthesis, like other instances of DNA replication, requires the formation of a 3′-OH primer terminus and an elongation reaction involving a DNA polymerase. Identification of participating enzymes has depended largely on measurement of DNA synthesis in conjugating temperature-sensitive (Ts) *dnaB*, *-E* and *-G* mutants of *E. coli*, exploiting the fact that a number of these mutants abruptly cease to support vegetative DNA replication at restrictive temperature. Since many of the pertinent investigations were reported before the enzymatic properties of these *dna* gene products were elucidated, this section will include a short survey of our understanding of relevant DNA replication proteins.

The *dnaE* product is the subunit and catalytically active component of the core of DNA polymerase III holoenzyme (103, 147), the primary replicative DNA polymerase of *E. coli*. Three enzymatic mechanisms for the generation of primers for this enzyme have been defined through studies of the requirements for DNA synthesis on the single-stranded, circular DNAs of small bacteriophages in the presence of single-strand binding protein. The first of these mechanisms involves RNA polymerase; this acts at a specific, highly base-paired region in the viral strand of phage M13 (or fd) to synthesize an RNA primer in a rifampin-sensitive process (31, 74, 76). The second and third priming mechanisms are rifampin resistant and both require primase, the 60,000-dalton product of the *dnaG* gene (139). On phage G4 DNA, primase catalyzes synthesis of an RNA or hybrid RNA-DNA primer that is initiated at a unique site on the viral strand

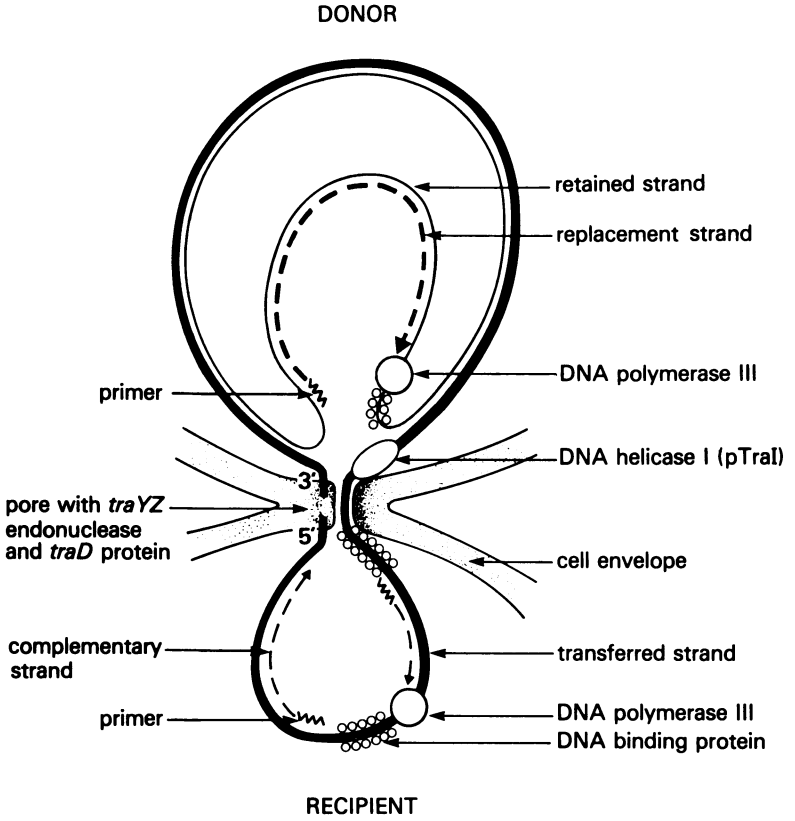

FIG. 4. Model for the conjugative transfer of F. A specific strand of the plasmid (thick line) is nicked at *oriT* by the *traYZ* endonuclease and transferred in the 5′ to 3′ direction through a pore, perhaps involving the *traD* protein, formed between the juxtaposed donor and recipient cell envelopes. The plasmid strand retained in the donor cell is shown by a thin line. The termini of the transferred strand are attached to the cell membrane by a complex that includes the endonuclease. DNA helicase I (*traI* product) migrates on the strand undergoing transfer to unwind the plasmid duplex DNA; if the helicase is in turn bound to the membrane complex during conjugation, the concomitant ATP hydrolysis might provide the motive force to displace the transferred strand into the recipient cell. DNA transfer is associated with synthesis of a replacement strand in the donor and of a complementary strand in the recipient cell (broken lines); both processes require de novo primer synthesis and the activity of DNA polymerase III holoenzyme. The model assumes that a single-strand binding protein coats DNA, to aid CDS; depending upon the nature of the pore, this protein might even be transferred from donor to recipient cell, bound to the DNA.

(140, 175). The other primase-dependent mechanism, utilized in complementary strand synthesis on φX174 DNA, requires a mobile, multiprotein complex termed the primosome which, in addition to primase, contains *E. coli* proteins i, n, n′, and n″ together with the *dnaB* and *dnaC* gene products (8). The primosome is believed to be assembled at a unique site on φX174 DNA, at or near the recognition site of protein n′, and from here the complex moves processively along the DNA template in the 5′ to 3′ direction to initiate synthesis of multiple primers at preferred sites (8, 11, 144). Utilization of these sites is thought to require the activity of *dnaB* protein to modify the secondary structure of the DNA for recognition by primase (9).

The mechanism described for the priming of φX174 complementary strand synthesis may function in lagging strand synthesis during replication of the *E. coli* chromosome and of some plasmids (103). A protein n′ (also designated factor Y) recognition site has been detected in each of the strands of ColE1 (125) and pBR322 (118, 146, 187), and short DNA segments containing signals for initiation of primosome-dependent DNA synthesis have been isolated from pDF31 (mini-F), pACYC177 (derived from the small multicopy plasmid p15A; 33), and CloDF13 (162). Comparison of the nucleotide sequences of the appropriate regions of these plasmids and φX174 DNA indicates that inverted repeat structures may be involved in protein n′ recognition and that the hexanucleotide 5′-AAGCGG-3′ may be the consensus sequence of part of the recognition element (118, 144, 162).

Replacement Strand Synthesis in the Donor Cell

DNA polymerase III holoenzyme is responsible for synthesis of DNA to replace the transferred strand of F, as indicated by the absence of DCDS during transfer of F *lac* from *dnaE486*(Ts) donor cells (99). Comparable studies have not been done with other plasmids, but detection of substantial DCDS in DNA polymerase I-deficient (*polA1*) cells (178) is consistent with participation of DNA polymerase III in the IncIα plasmid conjugation system too.

The rolling-circle model for DNA transfer predicts that the 3′-OH terminus of the DNA undergoing transfer serves to prime continuous synthesis of the replacement strand (78). However, the model may not be valid in view of indirect evidence that DCDS of both F and IncIα plasmids requires de novo synthesis of RNA primers, as reviewed below. In this context, it is notable that in vivo synthesis of viral strands of φX174 DNA from the replicative form, a process formally similar to plasmid transfer and DCDS, may also proceed discontinuously and require RNA priming (115, 121, 122, 152), even though studies with DNA polymerase III holoenzyme show that the 3′-OH terminus can be extended in vitro (60).

Investigations involving four different *dnaB*(Ts) alleles have established that transfer and DCDS of F (27, 28, 119, 166, 178), R64*drd-11* (65), and ColIb-P9*drd-1* (178) occur independently of *dnaB* protein and thus of the primosome. However, the thermoresistance of *dnaB* mutants may be enhanced or diminished by a variety of conjugative plasmids, including representatives of the IncFII and IncIα groups, and this led to the suggestion that plasmids carry *ban* (*dnaB* analog) genes which may act in conjugation (167, 168). Although search for one such *ban* protein was initiated (109), evidence favoring the presence of these genes on conjugative plasmids remains of a genetic nature (V. N. Iyer, personal communication).

The *dnaB* independence of DCDS has provided a convenient experimental system which can be used in conjunction with specific inhibitors or plasmid mutants to investigate the requirements for the process. The discovery that rifampin, but not an inhibitor of protein synthesis, blocked DCDS of F *lac* in *dnaB* cells while allowing plasmid transfer to proceed indicated that the strand undergoing transfer is not elongated efficiently from its 3′ terminus and suggested that untranslated RNA primes synthesis of the replacement strand (99). Furthermore, this result implies that DCDS of F cannot be initiated by *E. coli* primase acting either independently of primosome components or in conjunction with a putative *ban* protein. However, by analogy with RCDS (next section), it remains possible that more than one primer-generating process may function in wild-type cells and, whereas the primosome may provide one such mechanism, RNA polymerase substitutes effectively in its absence. Requirement for de novo synthesis of a primer contrasts with the ability of the 3′-OH terminus of nicked λ *oriT*$^+$ phage genomes to prime DNA synthesis by DNA polymerase I (see section, "Nicking at *oriT*"). Possibly packaging of the phage genome competes successfully with the sequestering reaction that makes this terminus unavailable in vivo.

Figure 3a shows that the nontransferred strand of F, which would act as template for the synthesis of an RNA primer(s), contains a potential promoter in the double-stranded region to the right of the *oriT* nicks as drawn (−35 sequence, nucleotides 255 to 250; −10 sequence, nucleotides 230 to 225). This putative promoter overlaps the long region of dyad symmetry mentioned earlier (see section, "Nicking at *oriT*"), although the significance of this is not known. After transfer has begun, DNA corresponding to the promoter for gene "X" located just to the left of the λ *oriT* nicks would be single stranded, and it is therefore unlikely that this could serve as template for primer synthesis by RNA polymerase. The hexanucleotide AAGCGG is not present in the 127 nucleotides to the left of the *oriT* nicks, suggesting that this region lacks a protein n′ recognition site, but one might be located yet further away beyond the *Bgl*II site where sequencing data stop. The putative protein n′ recognition site near nucleotides 233 to 238 (AAGCGG) is in a region that remains double stranded until the end of F DNA transfer, and it is not known whether this could play a role in initiating DCDS.

Although transfer of F normally occurs simultaneously with DCDS, it is not dependent upon this synthesis. This was first demonstrated in matings involving thymine-deprived Hfr *dnaB* donor cells at restrictive temperature (142) and was confirmed by the observations that *dnaE* or rifampin-treated *dnaB* donors of F *lac* transferred DNA efficiently at 42°C in the absence of DCDS (99). The converse situation has been reported since DCDS, but not DNA transfer, was detected at high temperature when *dnaB* cells containing F *traG* or *traN* mutants were added to potential recipient cells (99), and also when Hfr *dnaB* donors were mixed with F$^+$ minicells (134).

RNA primers have also been implicated in conjugative synthesis of IncIα plasmids. Models invoking RNA polymerase for synthesis of an untranslated RNA primer to initiate DCDS, and DNA transfer itself, stemmed from the finding that rifampin blocked both processes more effectively than chloramphenicol when *dnaB* donors of R64*drd-11* were mated with recipient minicells at 42°C (47, 66). However, in a subsequent study rifampin did not inhibit early transfer of ColIb-P9*drd-1* between *dna*$^+$ cells at 37°C and, since the drug was found to cause a general disruption of DNA metabolism in IncIα plasmid-containing bacteria, it was suggested that this was responsible for rifampin curtailing

transfer of these plasmids, rather than inhibition of the synthesis of an essential species of RNA (19). Irrespective of such experiments with rifampin, RNA synthesis in the donor cell must remain a candidate for initiating transfer or some other essential aspect of conjugative DNA metabolism, because inhibition of cytidine utilization in *dnaB* donors of R64*drd-11* was found to depress the yield of transconjugants (123). The affected enzyme might be the IncIα plasmid-encoded DNA primase (see below), since this synthesizes cytidine-containing RNA primers and enzyme supplied by the donor parent is involved in initiating CDS in both donor and recipient bacteria (35). Thus, as in the case of F, conjugative synthesis of an IncIα plasmid in the donor cell apparently requires de novo synthesis of a primer sequence, although different primer-generating mechanisms seem to be involved in the two conjugation systems.

Further studies of conjugative plasmids are clearly required, not only of the initial priming events but also of the continuity or otherwise of replacement strand synthesis which is assumed to be a continuous process in Fig. 4. There is a similar lack of information about the final stages of DCDS involving primer removal, gap filling, and ligation of the termini of the newly synthesized strand; presumably these stages involve bacterial enzymes such as DNA polymerase I and DNA ligase. The functions of these enzymes in DNA replication have been reviewed recently (102, 103, 129).

The small size of ColE1 and of other nonconjugative plasmids has precluded experiments to measure their synthesis during transfer, and hence the effects of *dna* mutations and rifampin on CDS of these plasmids are unknown. Most models for the transfer of ColE1 assume that the relaxation complex *nic* site is the origin of transfer, that the nicked H strand is transferred with a leading 5′ terminus, and that the 3′-OH terminus of this strand is elongated continuously by DCDS (148, 157, 172). However, if DCDS of ColE1 requires de novo synthesis of a primer, the necessary sequence might be made by a primosome formed at *rriA*. This locus, which is situated between *oriV* and *nic* in the L strand (Fig. 2 and 3b), contains a protein n′ recognition site and it is therefore implicated in primosome assembly. The site may function to initiate discontinuous synthesis of the H strand during vegetative replication (16, 125), although properties of pBR322 deletion mutants imply that it is not essential for this process (163). In conjugation, *rriA* might be left in a single-stranded form appropriate for primosome assembly and, allowing for migration of this multiprotein complex on single-stranded DNA, primer synthesis might occur between *rriA* and *nic*. Such a primer would allow continuous synthesis of a replacement strand in the donor cell, in the leftward direction in Fig. 2.

Complementary Strand Synthesis in the Recipient Cell

Conjugative DNA metabolism in the recipient involves two major events: synthesis of DNA complementary to the transferred strand and circularization of the plasmid DNA. Although both events may involve plasmid-encoded products supplied by the donor cell, neither requires expression of plasmid genes in the recipient cell because covalently closed circular duplex DNA can be formed in rifampin-treated recipients of either F (90) or ColIb-P9*drd-1* (20). Efficient mobilization and inheritance of plasmids carrying a cloned *oriT* site but no *tra* genes provide further confirmation (62). RCDS has proved more amenable to study than the corresponding process in the donor because it can be detected in cells heavily irradiated with UV light to render resident DNA unusable as a template for DNA synthesis (72). This procedure allows measurement of RCDS both in *dna*$^+$ strains and in those temperature-sensitive *dna* mutants that continue to synthesize a significant amount of DNA even at the restrictive temperature. Use of this approach in conjunction with R1*drd-19* (IncFII) has shown that the transferred DNA is initially replicated in association with the membrane of the recipient cell to give linear double-stranded molecules, and these are then converted into open and covalently closed circular forms present in the cytoplasm (64). That RCDS precedes circularization (Fig. 4) is assumed also to be true for other conjugative plasmids, but it may not be true for nonconjugative plasmids (see below).

Conjugative synthesis of F *lac* and ColIb-P9*drd-1* in recipient cells requires *dnaE* product and is presumably mediated by DNA polymerase III holoenzyme (178), but different primer-generating mechanisms are used in the two conjugation systems. In the case of F-prime plasmids, RCDS has been detected at restrictive temperature in recipients carrying the *dnaB266* or *dnaB70* allele (27, 178). However, conflicting results were obtained with *dnaB43* mutants since such recipient cells failed to support RCDS in one study (27), whereas synthesis of DNA and formation of covalently closed circular plasmids apparently occurred at restrictive temperature in another *dnaB43* strain of different background genotype (166). Since *dnaG3*(Ts) mutants also supported RCDS of F *lac* (18), it may be inferred that neither the primosome nor the primase of the recipient cell is essential for initiating DNA synthesis on the transferred strand of F. Like DCDS, RCDS of F *lac* was found to be rifampin sensitive in *dnaB* mutants, but the process was resistant to the drug in *dna*$^+$ recipients (178). These observations may be reconciled if it is assumed that primers for synthesis of DNA complementary to the transferred strand of F may be made by either RNA polymerase or the activity of the primosome, but not by primase alone, and that when one of these systems is inactivated, the other can substitute effectively. No promoter or protein n′ recognition site (AAGCGG) is apparently present in the first 127 nucleotides of F DNA to be transferred, which is the extent of the available sequence data (Thompson et al., submitted).

In contrast to F, none of the three defined priming mechanisms specified by *E. coli* is essential for initiating conversion of the transferred strand of an IncIα plasmid to double-stranded DNA, since RCDS proceeded in *dnaB* and *dnaG* recipient cells and, in both mutants, it was rifampin resistant (20, 21, 178). The possibility that the donor cell provides *dnaG* protein or RNA polymerase for this process may be discounted (21). It is now apparent that conjugative synthesis of an IncIα plasmid in the recipient is initiated primarily by the activity of a plasmid-encoded DNA primase supplied by the donor cell (see next section).

It is not known whether complementary strand synthesis in the recipient cell is a continuous or discontinuous process, but Fig. 4 assumes discontinuous synthesis involving formation of multiple primer sequences. Although this is the more complex mechanism, it has the appealing property of minimizing accumulation of single-stranded DNA in the recipient cell, and thus reducing the potential vulnerability of the transferred DNA to irreparable damage such as single-strand breaks. In common with DCDS, there is a scarcity of information about the final stages involving primer removal and ligation of the termini of the newly synthesized DNA.

Several studies pertain to the priming of RCDS of ColE1 and of other nonconjugative plasmids. The primosome is the most likely candidate for initiating conjugative synthesis of

ColE1 in the recipient cell since the ColE1 H strand, which is thought to be the conjugatively transferred strand (172), contains a locus called *rriB* that can promote rifampin-resistant initiation of DNA synthesis by a process dependent on *E. coli* proteins constituting the primosome (16, 125). The *rriB* site, which is recognized in vitro by protein n′ and may therefore serve as the assembly site of the primosome, lies in the *Hae*II-C fragment of ColE1 (16, 125, 187; Fig. 2). This fragment also contains *nic*. Since ColE1 deletion mutants lacking this fragment can be isolated (131, 132, 160, 172), *rriB* is not essential for vegetative DNA replication, fostering the view that the site acts as the origin of complementary strand synthesis in the recipient cell. The same suggestion has stemmed from parallel studies of the protein n′ recognition site on the H strand of the *Hae*II-B fragment of pBR322 (188), this site likewise being inessential for plasmid replication (163). Since *rriB* is one of the last sequences to be transferred, RCDS would be initiated at the completion of ColE1 transfer, presumably after circularization of the transferred strand, rather than by multiple priming events during DNA transfer as we have assumed for large conjugative plasmids. It is interesting that no initiation signal for primosome-dependent DNA synthesis was detected in an 850-bp fragment of CloDF13 spanning the origin of transfer (162). Thus, RCDS of this plasmid may be initiated at a different location or by a different mechanism from that used in ColE1 transfer.

Alternatively, RCDS of ColE1 might be initiated by RNA polymerase, possibly acting from the cyclic AMP receptor protein-dependent promoter close to *nic* (137; Fig. 2 and 3b). However, there is no inverted repeat sequence in this region to provide the necessary double-stranded DNA substrate, and this promoter may instead be used for transcription of the nearby *mob* genes (68).

The striking proximity of *nic* and the origin of vegetative DNA replication (*oriV*) in a number of plasmids including ColE1, pSC101, RSF1010 (IncQ), and the conjugative plasmid R6K (IncX), where there is a *nic* site close to each of two *oriV* sites, inspired the suggestion that the two sites are functionally related during conjugative transfer (127). The essential features of the model are that DNA synthesis is initiated at *oriV* in the donor cell to proceed towards *nic* on the strand destined for transfer and that this newly synthesized DNA sequence is transmitted with the transferred strand to the recipient where it acts to prime RCDS. However, two recent findings cast doubt on the validity of this model. First, cloning experiments with derivatives of ColE1, which replicates unidirectionally (93, 113, 159), have shown that the position and, more significantly, the orientation of *nic* relative to *oriV* can be changed without affecting conjugative mobility (69). In the case of the constructs with an inverted *nic* site, the hypothetical DNA primer would be complementary to the nontransferred strand, and it would remain in the donor cell. The reason for apparently contradictory findings with pMB1 derivatives (46) is not clear. Second, RP4-mediated mobilization of R300B (which is similar to RSF1010) seemingly requires the DNA primase encoded by the conjugative plasmid for efficient RCDS in certain genera (108); involvement of such an enzyme in this process would not be expected if it were initiated by the putative DNA primer.

Single-Stranded DNA Binding Proteins

By analogy with phage and bacterial DNA replication, conjugative DNA synthesis is likely to require a single-stranded DNA binding protein (SSB). The *E. coli* SSB protein binds cooperatively to single-stranded DNA, destabilizes duplex DNA, and is required in vivo for DNA replication (see references 75, 102, 103, 174). More pertinent to conjugative DNA metabolism, it acts during in vitro synthesis of the complementary strand on DNA of phages M13, G4, and φX174 to promote correct initiation and to enhance chain elongation by DNA polymerase III holoenzyme. Involvement of SSB protein in DCDS and RCDS has yet to be examined and temperature-sensitive *ssb* mutants (79) will be useful in this regard.

Some conjugative plasmids specify their own single-stranded DNA binding proteins. Plasmid F and representatives of the FII and Iα incompatibility groups partially reverse the temperature sensitivity of *E. coli ssb-1* mutants, and the F product responsible for this suppression is known to be a single-stranded DNA binding protein (101; E. Golub and B. Low, personal communication). This protein has extensive homology with SSB and a molecular weight calculated from the DNA sequence of 19,505 (34). It is encoded by a gene, denoted *ssf*, located at about coordinate 59.5 on our map (Fig. 1) in a segment of F that is outside the transfer region and transferred early during conjugation (101). Suppression of *ssb-1* by R64 (IncIα), but not by R1 or R100 (IncFII), was dependent on a *drd* mutation in the plasmid, and since such mutations derepress expression of transfer genes, the putative single-stranded DNA binding protein of IncIα plasmids may participate in conjugative DNA metabolism. If the protein functions in RCDS, it would presumably be transported from the donor cell because this DNA synthesis does not require expression of genes on the transferred plasmid strand, as discussed earlier.

Plasmid DNA Primases

A variety of plasmids specify DNA primases, and these enzymes are thought to generate primers for conjugative DNA synthesis. Existence of plasmid DNA primases was first suggested by the discovery that IncIα plasmids can partially suppress the effect of temperature-sensitive *dnaG* mutations in *E. coli* (176), and it was demonstrated directly by the purification of a novel primer-generating enzyme from bacteria harboring R64*drd-11* (109). Subsequent studies including ColIb-P9*drd-1* and R144*drd-3* have shown that all three IncIα plasmids contain homologous primase genes (51) and that they specify synthesis of the same enzyme as judged by immunological cross-reaction and electrophoretic mobility of plasmid products (52, 177). The primases encoded by this group of plasmids will therefore be considered together.

IncIα plasmid primase, which is antigenically distinct from *E. coli* primase (177), can utilize a surprising diversity of templates both in vitro and in vivo. The enzyme can act on viral strands of phages fd (and M13), G4, and φX174, substituting for the activities of *E. coli* RNA polymerase, *dnaG* protein, and at least the *dnaB*, *dnaC*, and *dnaG* protein components of the primosome, respectively (109). In vivo the enzyme can substitute for *E. coli* primase during discontinuous DNA replication, allowing, in the case of *dnaG3*(Ts) mutants, extensive colony formation at high temperature and replication of phage λ DNA and IncIα and ColE1-like plasmids (176, 177).

The primase of IncIα plasmids acts in a rifampin-resistant process to make RNA primers that are utilized both in vitro and in vivo by DNA polymerase III holoenzyme (109, 177). Functional primers made in vitro range in size from 2 to about 10 nucleotides and, in contrast to those made by *E. coli* primase which have a purine in the initiating nucleotide (10, 140), they contain cytidine or CMP at the 5′ terminus

with AMP as the second nucleotide (E. Lanka, personal communication). The enzyme can recognize a 3'-dGdT-5' sequence located at the end as well as within a polydeoxyribonucleotide strand; ability to use the 3'-terminal nucleotide in a linear template, an exceptional property for a primer-generating enzyme, may prove to be a significant attribute for the proposed role of the enzyme in conjugative DNA synthesis.

The ColIb-P9 primase locus, termed *sog*, specifies two polypeptides of 240,000 and 180,000 apparent molecular weights, and as these are immunologically cross-reactive, they share a common amino acid sequence. These proteins are thought to be generated from a single transcript by in-phase translation from two initiation sites (22, 177). Only the larger polypeptide has DNA primase activity, and it may be multifunctional since properties of amber and deletion mutants have shown that primase activity requires only the amino-terminal one-third or so of this protein; indeed, some truncated forms of this polypeptide are more effective than the wild-type protein in promoting bacterial DNA replication in a *dnaG* mutant of *E. coli* (22, 177). Although R64*drd-11* primase activity was initially reported to be associated with a 140,000-dalton polypeptide (109), which was subsequently shown to react with antiserum raised against ColIb-specified DNA primase, a protein of this size cannot be detected in crude cell extracts using an immunoassay, and it may have been formed during the purification process by degradation of a larger molecule (Lanka, personal communication).

Table 2 shows that a variety of other groups of plasmids have the potential to suppress the *dnaG3* mutation, and in general, these also specify a DNA primase activity. The assay used so far has measured the ability of crude cell extracts to stimulate DNA synthesis on viral strands of phage fd or M13 in the presence of rifampin. Although a wide range of plasmids are currently classified as primase negative (108), it is possible that some may specify priming enzymes that are not detectable in this assay because they possess a greater template specificity.

TABLE 2. Plasmids with the potential to suppress the *E. coli dnaG3* mutation and to specify DNA primase[a]

Representative plasmid(s)	Inc group[b]	Suppression of *dnaG3* (reference)[c]	Primase activity (reference)[c]
R16, TP125[d]	B	+ (51)	+ (51, 108)
R864a, RIP72[e]	B	+ (51)	+ (52)
R40a	C	+ (50)	+ (50)
ColIb-P9, R64[e]	Iα	+ (143, 176)	+ (109, 177)
TP114	I2	Not known	+ (108)
R621a[e]	Iγ(B)	+ (51, 143)	+ (108)
R805a[d]	Iζ	+ (51)	+ (50)
R391	J	+ (50, 143)	− (50)
R387[d]	K	+ (50)	+ (50, 108)
R831b	M	− (50)	+ (108)
RP4, R68 · 45	P	+ (108, 114)	+ (108)
RA3	U	+ (50)	+ (50)

[a] Note that detection of one or both of these properties may require mutants of the plasmid.

[b] IncIα, -Iγ, -Iζ, -B, and -K plasmids show significant DNA homologies and specify serologically related pili (see reference 41 for a recent summary). R621a has been designated an IncIγ plasmid (Appendix B in reference 32) but it is incompatible with IncB plasmids (41).

[c] +, Detected; −, not detected.

[d] Plasmids on these lines contain nucleotide sequences homologous with the R16 primase gene (50, 51).

[e] Plasmids on these lines contain nucleotide sequences homologous with the ColIb-P9 primase gene (51).

At least three different plasmid DNA primases have been found, the prototypes of which are encoded by ColIb-P9, R16(IncB), and RP4(IncP). The primase genes of ColIb-P9 and R16 are distinguishable by DNA hybridization tests and their products are antigenically distinct (51). However, the organization of the two genes may be the same because the *pri* gene of R16 specifies two polypeptides similar in size to the products of the ColIb-P9 *sog* gene (50). IncB, -Iα, -Iζ, and -K plasmids, which show significant levels of polynucleotide sequence homology (41, 63, 80), can be separated into two sets depending on whether they contain sequences homologous with the cloned primase genes of ColIb-P9 or R16 (50, 51), but these sets (Table 2) do not correlate with other groupings of these plasmids based on incompatibility (41, 53), pilus serotype (24), or host range of plasmid-dependent bacteriophages (41).

RP4 encodes a primase that is immunologically distinct from the enzymes specified by the ColIb-P9 *sog* gene and the *E. coli dnaG* gene (108). Moreover, the *pri* genes of RP4 and R16 differ since the cloned R16 determinant does not hybridize with RP4 (50). The RP4 *pri* gene directs synthesis of two antigenically related polypeptides of 118,000 and 80,000 daltons, and unlike the products of *sog*, both proteins possess primase activity (108). Despite these differences, RP4 and IncIα plasmid primases are similar with regard to templates that can be utilized in vitro (108), the products of their reactions, and also their ability (requiring enzyme overproduction in the case of RP4 primase) to promote bacterial DNA replication in a *dnaG3* strain of *E. coli* at restrictive temperature (Lanka, personal communication).

Primase-defective mutants of RP4 (108) and a ColIb-P9*drd-1* derivative (35) have been isolated and used to examine the function of this class of enzyme. Plasmid maintenance studies indicated that the primase specified by ColIb-P9 is inessential for vegetative plasmid replication, although the RP4 enzyme may make a small contribution to this process. However, both types of mutant showed some deficiency in conjugation, implying that plasmid DNA primases act in conjugative DNA metabolism. This is consistent with the observation that the primase genes of IncIα plasmids (108, 176, 177), R621a (IncIγ/B; 143), and R864a (IncB; 52) are coordinately regulated with the transfer genes and also with the suggestion that the RP4 *pri* gene constitutes part of the TraI region of the plasmid (108).

The DNA primase of RP4 was required for efficient conjugation in certain intergeneric matings (108). Whereas the yield of transconjugants was normal when *E. coli* donors of the *pri* mutants were mated with six recipient species, including *E. coli* and *Pseudomonas* spp., it was depressed by up to 95% in matings with five other species, including *Salmonella typhimurium*. Since this effect depended on the species used as the recipient rather than the donor, it was postulated that the enzyme participates in the priming of DNA synthesis on the transferred plasmid strand and that this activity is necessary for the formation of transconjugants in a bacterial species producing a DNA primase that does not efficiently recognize priming sites on RP4.

The primase-defective mutants of ColIb-P9*drd-1* were about 80% deficient in conjugation in both *E. coli* and *S. typhimurium* (35). It was inferred from measurements of conjugative DNA synthesis that, although the plasmid enzyme is not required for DNA transfer itself, it participates in the donor cell to initiate synthesis of DNA to replace the transferred strand, and it acts to prime synthesis of the

complementary strand in the recipient. Primase encoded by the *dnaG* gene of the recipient cell can function in the latter process with low efficiency, thus explaining the partial conjugation proficiency of the mutant plasmids.

Initiation of complementary strand synthesis in the recipient by plasmid primase is thought to be mediated by enzyme supplied by the donor parent. This hypothesis (21) received strong support from the demonstration that a non-mobilizable Sog^+ recombinant plasmid in the donor could complement a primase-defective mutant of ColIb-P9*drd-1* and stimulate RCDS (35). The primase, which binds to single-stranded DNA (109), is apparently transferred between conjugating bacteria because *dnaG3* recipient cells, treated with rifampin to inhibit transcription of the transferred DNA, acquired limited ability to synthesize chromosomal DNA during a mating at restrictive temperature with donors containing an IncIα plasmid (L. Chatfield and B. Wilkins, unpublished data). Therefore, primers might be made in the donor and transferred with the DNA and enzyme, or they might be synthesized in the recipient cell after enzyme transfer.

It is not clear why some conjugative plasmids encode a DNA primase while others may not. Clarification will require a greater understanding of these large proteins and in particular of the role of the domain that is inessential for primase activity. It is apparent that the transferred DNA of IncIα or IncP plasmids is not utilized efficiently by the priming enzymes of certain gram-negative bacteria, even though the host enzymes presumably function to initiate discontinuous replication of these plasmids. Possibly the cellular location or the conformation of the transferred strand is inappropriate for the activity of host enzymes, or the recognition sites are masked by a product, such as a plasmid-encoded single-strand binding protein, which acts to optimize some other aspect of conjugative DNA metabolism.

CIRCULARIZATION OF TRANSFERRED DNA

The rolling-circle model for DNA transfer (78) hypothesized transfer of linear single-stranded DNA of greater than unit length, and this raised the possibility that circularization occurs in the recipient cell by recombination between homologous DNA regions. Even though the *recA* independence of plasmid inheritance (35) showed that a host-encoded recombination mechanism is not required, a plasmid-encoded system might substitute (but see below). Two early investigations of F- and F-prime-mediated conjugation showed that the recipient cells accumulated DNA that sedimented more rapidly than linear plasmid monomers, leading to the suggestion that plasmid strands of greater than unit length are indeed transferred (120, 130). However, the relative sedimentation rates of linear and circular forms of the plasmids were not determined, and it is possible that the transferred DNA was in fact in the form of monomeric circles. The latter interpretation is favored by results of subsequent studies with F and R1*drd-19*, indicating that the plasmid DNA recovered from recipient cells was predominantly in the form of monomeric molecules, with up to one-half being covalently closed circles of unit length (64, 90, 165, 166). Similarly, transferred ColIb-P9*drd-1* DNA was found in the form of circular and linear monomers, even when the cells were mated under conditions that impeded termination of conjugation and allowed transfer of about five plasmid strands from each donor cell (20). Although none of these experiments rule out the possibility of transfer of concatemeric DNA and its rapid cleavage into monomeric molecules, such a model is inconsistent with the detection of a delay of several minutes between first-round transfer of R64*drd-11* DNA to minicells and second-round transfer of the newly synthesized replacement strand (65) and of a requirement for protein synthesis in the donor cells for initiation of second and subsequent rounds of plasmid transfer (66). Furthermore, the discovery that de novo synthesis of a primer is apparently required in donor cells to initiate synthesis of DNA to replace the transferred strand of F *lac* (99) or ColIb-P9*drd-1* (35) implies that the 3′ terminus of a strand undergoing transfer cannot be extended, in contrast to the prediction of the rolling-circle model.

We therefore conclude that plasmids are transferred as discrete strands of unit length. If so, a mechanism must exist for the precise religation of the 5′ and 3′ termini at *oriT*. Involvement of a plasmid-encoded system expressed after DNA transfer is unlikely since circularization of transferred F and ColIb-P9*drd-1* DNA occurs in the absence of plasmid gene expression in the recipient cell (20, 62, 90). An attractive possibility is a process analogous to that described for circularization of monomeric lengths of ϕX174 DNA by the gene *A* endonuclease-ligase during virus multiplication (59). Thus, upon nicking at *oriT*, the 5′ terminus would be covalently linked to a membrane protein, perhaps conserving the energy of the cleaved phosphodiester bond, and after completion of DNA transfer, this protein would recognize the 3′ terminus (possibly also protein bound) and ligate it to the 5′ terminus. The *traYZ* endonuclease is a candidate for this protein in F transfer (61). A similar mechanism has been proposed for circularization of transferred ColE1 DNA; in this case the process is postulated to involve the 60,000-dalton protein of the relaxation complex (172). Circularization of transferred DNA by a mechanism that ligates the two termini at *oriT* is supported by the discovery that ColE1 and related plasmids can form cointegrates by a *recA*-independent process after their mobilization from the same donor cell and that the component plasmids in such cointegrates can separate during further conjugative transfer, again by a mechanism that is independent of *recA* protein (29, 169). The site of cointegration was found to lie at or within 47 bp of the ColE1 relaxation complex nick site (29), and separation also required the presence of two *nic* sites (169). Thus, cointegration may result from ligation of the 5′ terminus of one transferred molecule to the 3′ terminus of another, and vice versa. Separation may occur if both *oriT* sites in a cointegrate are nicked in the donor cell, or if only one site is nicked before transfer and the other is recognized and nicked as it enters the recipient cell. Conjugative dissociation of plasmid multimers is not restricted to ColE1-related plasmids since a tetramer of a chimeric plasmid containing the F *oriT* on a ca. 385-bp fragment was very efficiently monomerized after its mobilization by F *lac* between *recA* strains (62).

Circularization of the transferred strand provides a continuous covalently closed template which is required for the completion of complementary strand synthesis. After removal of RNA primers and ligation of the termini of the newly synthesized DNA, a topoisomerase, such as DNA gyrase, presumably introduces negative superhelical turns to generate the plasmid forms necessary for efficient transcription and the initiation of vegetative DNA replication.

CONCLUSIONS

It is apparent from this review that our current perspective of conjugation is dominated by knowledge of IncF and, to a lesser extent, IncI plasmids. However, despite the very sophisticated information available on the organization of

the F transfer genes and their general role in the transfer process, much remains to be learned about the biochemistry of the *tra* gene products involved in conjugative DNA metabolism. The activity of only one, the DNA unwinding protein specified by *traI*, has been characterized in vitro, and analogous studies of the others, including the *traYZ* endonuclease and the *traD* and *traM* proteins, remain to be carried out. In addition to plasmid-specified products, bacterial proteins are required for synthesis of RNA primers and for conjugative DNA synthesis itself. The molecular interactions between plasmid and host proteins and their DNA substrates for the interconnected processes of nicking at *oriT*, triggering of conjugation, unwinding of DNA, and synthesis of RNA primers require further clarification. Also, the contributions to primer synthesis in wild-type donor cells of RNA polymerase, the primosome, and (in some cases) plasmid-specified primase have yet to be evaluated. The novel concept that some proteins that play a role in conjugative DNA metabolism in the recipient cell, such as plasmid primase, *tra* membrane proteins, and perhaps single-strand binding protein, are physically transferred from the donor cell warrants further investigation.

In summary, we anticipate that two approaches will be particularly rewarding in future research. First, there is a need to characterize the fundamental aspects of plasmid DNA metabolism in a range of different conjugation systems. Comprehension of the conjugative metabolism of large plasmids other than F is fragmentary, as is understanding of the molecular interactions involved in the mobilization of small nonconjugative plasmids. Indeed, the central tenet of many models of plasmid transfer, transmission of a specific single strand with a leading 5′ terminus, has only been proven for F. These studies are therefore required to allow both definition of general principles and recognition of the diversity of molecular interactions that may occur. Second, the development of in vitro systems to study the enzymology of conjugative transfer and synthesis of plasmid DNA is likely to be invaluable. Analysis of the details of these processes through in vivo studies is technically challenging because only a minority of the DNA in cells is transferred, and detection and identification of intermediates are further complicated by the cell-to-cell interactions necessary for initiation and continuation of DNA transfer.

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LITERATURE CITED

1. **Abdel-Monem, M., and H. Hoffmann-Berling.** 1976. Enzymatic unwinding of DNA. I. Purification and characterization of a DNA-dependent ATPase from *Escherichia coli*. Eur. J. Biochem. **65**:431–440.
2. **Abdel-Monem, M., H.-F. Lauppe, J. Kartenbeck, H. Dürwald, and H. Hoffmann-Berling.** 1977. Enzymatic unwinding of DNA. III. Mode of action of *Escherichia coli* DNA unwinding enzyme. J. Mol. Biol. **110**:667–685.

2a. **Abdel-Monem, M., G. Taucher-Scholz, and M.-Q. Klinkert.** 1983. Identification of *Escherichia coli* DNA helicase I as the *traI* gene product of the F sex factor. Proc. Natl. Acad. Sci. U.S.A. **80**:4659–4663.

3. **Achtman, M., P. A. Manning, C. Edelbluth, and P. Herrlich.** 1979. Export without proteolytic processing of inner and outer membrane proteins encoded by F sex factor *tra* cistrons in *Escherichia coli* minicells. Proc. Natl. Acad. Sci. U.S.A. **76**:4837–4841.
4. **Achtman, M., G. Morelli, and S. Schwuchow.** 1978. Cell-cell interactions in conjugating *Escherichia coli*: role of F pili and fate of mating aggregates. J. Bacteriol. **135**:1053–1061.
5. **Achtman, M., R. A. Skurray, R. Thompson, R. Helmuth, S. Hall, L. Beutin, and A. J. Clark.** 1978. Assignment of *tra* cistrons to *Eco*RI fragments of F sex factor DNA. J. Bacteriol. **133**:1383–1392.
6. **Al-Doori, Z., M. Watson, and J. Scaife.** 1982. The orientation of transfer of the plasmid RP4. Genet. Res. **39**:99–103.
7. **Alfaro, G., and N. Willetts.** 1972. The relationship between the transfer systems of some bacterial plasmids. Genet. Res. **20**:279–289.
8. **Arai, K., and A. Kornberg.** 1981. Unique primed start of phage ϕX174 DNA replication and mobility of the primosome in a direction opposite chain synthesis. Proc. Natl. Acad. Sci. U.S.A. **78**:69–73.
9. **Arai, K., and A. Kornberg.** 1981. Mechanism of *dnaB* protein action. III. Allosteric role of ATP in the alteration of DNA structure by *dnaB* protein in priming replication. J. Biol. Chem. **256**:5260–5266.
10. **Arai, K., and A. Kornberg.** 1981. Mechanism of *dnaB* protein action. IV. General priming of DNA replication by *dnaB* protein and primase compared with RNA polymerase. J. Biol. Chem. **256**:5267–5272.
11. **Arai, K., R. L. Low, and A. Kornberg.** 1981. Movement and site selection for priming by the primosome in phage ϕX174 DNA replication. Proc. Natl. Acad. Sci. U.S.A. **78**:707–711.
12. **Barbour, S. D.** 1967. Effect of nalidixic acid on conjugational transfer and expression of episomal *Lac* genes in *Escherichia coli* K12. J. Mol. Biol. **28**:373–376.
13. **Bastia, D.** 1978. Determination of restriction sites and the nucleotide sequence surrounding the relaxation site of ColE1. J. Mol. Biol. **124**:601–639.
14. **Blair, D. G., D. B. Clewell, D. J. Sherratt, and D. R. Helinski.** 1971. Strand-specific supercoiled DNA-protein relaxation complexes: comparison of the complexes of bacterial plasmids $ColE_1$ and $ColE_2$. Proc. Natl. Acad. Sci. U.S.A. **68**:210–214.
15. **Blair, D. G., and D. R. Helinski.** 1975. Relaxation complexes of plasmid DNA and protein. I. Strand-specific association of protein and DNA in the relaxed complexes of plasmids ColE1 and ColE2. J. Biol. Chem. **250**:8785–8789.
16. **Böldicke, T. W., G. Hillenbrand, E. Lanka, and W. L. Staudenbauer.** 1981. Rifampicin-resistant initiation of DNA synthesis on the isolated strands of ColE plasmid DNA. Nucleic Acids Res. **9**:5215–5231.
17. **Bouck, N., and E. A. Adelberg.** 1970. Mechanism of action of nalidixic acid on conjugating bacteria. J. Bacteriol. **102**:688–701.
18. **Boulnois, G. J.** 1980. Studies on the transfer of IncIα plasmids in *Escherichia coli* K-12. Ph.D. thesis, University of Leicester, Leicester, England.
19. **Boulnois, G. J., M. J. Beddoes, and B. M. Wilkins.** 1979. Rifampin disrupts conjugal and chromosomal deoxyribonucleic acid metabolism in *Escherichia coli* K-12 carrying some Incα plasmids. J. Bacteriol. **138**:324–332.
20. **Boulnois, G. J., and B. M. Wilkins.** 1978. A ColI-specified product, synthesized in newly infected recipients, limits the amount of DNA transferred during conjugation of *Escherichia coli* K-12. J. Bacteriol. **133**:1–9.
21. **Boulnois, G. J., and B. M. Wilkins.** 1979. A novel priming system for conjugal synthesis of an Incα plasmid in recipients. Mol. Gen. Genet. **175**:275–279.
22. **Boulnois, G. J., B. M. Wilkins, and E. Lanka.** 1982. Overlapping genes at the DNA primase locus of the large plasmid ColI. Nucleic Acids Res. **10**:855–869.
23. **Bradley, D. E.** 1978. W pili: characteristics and interaction with lipid phages specific for N, P-1, and W group plasmids, p. 339–353. *In* D. E. Bradley, E. Raizen, P. Fives-Taylor, and J. Ou (ed.), Pili. International Conferences on Pili, Washington, D.C.

24. **Bradley, D. E.** 1980. Morphological and serological relationships of conjugative pili. Plasmid **4**:155–169.
25. **Bradley, D. E.** 1981. Conjugative pili of plasmids in *Escherichia coli* K-12 and *Pseudomonas* species, p. 217–226. *In* S. B. Levy, R. C. Clowes, and E. L. Koenig (ed.), Molecular biology, pathogenicity, and ecology of bacterial plasmids. Plenum Press, New York.
26. **Bradley, D. E., and T. Chaudhuri.** 1980. Characteristics and interaction with bacteriophages of pili determined by a plasmid of the N incompatibility group, p. 335–342. *In* C. Stuttard and K. R. Rozee (ed.), Plasmids and transposons. Academic Press, Inc., New York.
27. **Bresler, S. E., V. A. Lanzov, and V. T. Likhachev.** 1973. On the mechanism of conjugation in *Escherichia coli* K12. III. Synthesis of DNA in the course of bacterial conjugation. Mol. Gen. Genet. **120**:125–131.
28. **Bresler, S. E., V. A. Lanzov, and A. A. Lukjaniec-Blinkova.** 1968. On the mechanism of conjugation in *Escherichia coli* K12. Mol. Gen. Genet. **102**:269–284.
29. **Broome-Smith, J.** 1980. *RecA* independent, site-specific recombination between ColE1 or ColK and a miniplasmid they complement for mobilization and relaxation: implications for the mechanism of DNA transfer during mobilization. Plasmid **4**:51–63.
30. **Brown, A. M. C., and N. S. Willetts.** 1981. A physical and genetic map of the IncN plasmid R46. Plasmid **5**:188–201.
31. **Brutlag, D., R. Schekman, and A. Kornberg.** 1971. A possible role for RNA polymerase in the initiation of M13 DNA synthesis. Proc. Natl. Acad. Sci. U.S.A. **68**:2826–2829.
32. **Bukhari, A. I., J. A. Shapiro, and S. L. Adhya.** 1977. DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
34. **Chase, J. W., B. M. Merrill, and K. R. Williams.** 1983. F sex factor encodes a single-stranded DNA binding protein with extensive homology to *Escherichia coli* SSB. Proc. Natl. Acad. Sci. U.S.A. **180**:5480–5484.
35. **Chatfield, L. K., E. Orr, G. J. Boulnois, and B. M. Wilkins.** 1982. DNA primase of plasmid ColIb is involved in conjugal DNA synthesis in donor and recipient bacteria. J. Bacteriol. **152**:1188–1195.
36. **Clark, A. J.** 1967. The beginning of a genetic analysis of recombination-proficiency. J. Cell. Physiol. **70**(Suppl. 1):165–180.
37. **Clewell, D. B.** 1981. Conjugation and resistance transfer in streptococci and other Gram positive species: plasmids, sex pheromones and "conjugative transposons" (a review), p. 191–205. *In* S. B. Levy, R. C. Clowes, and E. L. Koenig (ed.), Molecular biology, pathogenicity, and ecology of bacterial plasmids. Plenum Press, New York.
38. **Clewell, D. B., and D. R. Helinski.** 1969. Supercoiled circular DNA-protein complex in *Escherichia coli*: purification and induced conversion to an open circular DNA form. Proc. Natl. Acad. Sci. U.S.A. **62**:1159–1166.
39. **Clewell, D. B., and D. R. Helinski.** 1970. Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry **9**:4428–4440.
40. **Clewell, D. B., and D. R. Helinski.** 1970. Existence of the colicino-genic factor-sex factor $ColI_b$-P9 as a supercoiled circular DNA-protein relaxation complex. Biochem. Biophys. Res. Commun. **41**:150–156.
41. **Coetzee, J. N., D. E. Bradley, and R. W. Hedges.** 1982. Phages Iα and I_2-2: IncI plasmid-dependent bacteriophages. J. Gen. Microbiol. **128**:2797–2804.
42. **Cohen, A., W. D. Fisher, R. Curtiss III, and H. Adler.** 1968. The properties of DNA transferred to minicells during conjugation. Cold Spring Harbor Symp. Quant. Biol. **33**:635–640.
43. **Cohen, A., W. D. Fisher, R. Curtiss III, and H. I. Adler.** 1968. DNA isolated from *Escherichia coli* minicells mated with F^+ cells. Proc. Natl. Acad. Sci. U.S.A. **61**:61–68.
44. **Collins, J.** 1979. Cell-free synthesis of proteins coding for mobilization functions ColE1 and transposition functions of Tn*3*. Gene **6**:29–42.
45. **Collins, J., S. Yanofsky, and D. R. Helinski.** 1978. Altered DNA-protein relaxation complex in a replication mutant of plasmid ColE1. Mol. Gen. Genet. **167**:21–28.
46. **Covarrubias, L., L. Cervantes, A. Covarrubias, X. Soberon, I. Vichido, A. Blanco, Y. M. Kupersztoch-Portnoy, and F. Bolivar.** 1981. Construction and characterization of new cloning vehicles. V. Mobilization and coding properties of pBR322 and several deletion derivatives including pBR327 and pBR328. Gene **13**:25–35.
47. **Curtiss, R., III, and R. G. Fenwick, Jr.** 1975. Mechanism of conjugal plasmid transfer, p. 156–165. *In* D. Schlessinger (ed.), Microbiology—1974. American Society for Microbiology, Washington, D.C.
48. **Curtiss, R., III, R. G. Fenwick, Jr., R. Goldschmidt, and J. O. Falkinham III.** 1977. Mechanism of conjugation, p. 109–134. *In* S. Mitsuhashi (ed.), R. factor, drug resistance plasmid. University Park Press, Baltimore.
49. **Cuzin, F., G. Buttin, and F. Jacob.** 1967. On the mechanism of genetic transfer during conjugation of *Escherichia coli*. J. Cell. Physiol. **70**(Suppl. 1):77–88.
50. **Dalrymple, B. P.** 1982. Plasmid encoded DNA primases. Ph.D. thesis, University of Leicester, Leicester, England.
51. **Dalrymple, B. P., G. J. Boulnois, B. M. Wilkins, E. Orr, and P. H. Williams.** 1982. Evidence for two genetically distinct DNA primase activities specified by plasmids of the B and I incompatibility groups. J. Bacteriol. **151**:1–7.
52. **Dalrymple, B. P., and P. H. Williams.** 1982. Detection of primase specified by IncB plasmid R864a. J. Bacteriol. **152**:901–903.
53. **Datta, N.** 1979. Plasmid classification: incompatibility grouping, p. 3–12. *In* K. N. Timmis and A. Pühler (ed.), Plasmids of medical, environmental and commercial importance. Elsevier/North-Holland Biomedical Press, Amsterdam.
54. **Datta, N., and P. T. Barth.** 1976. Hfr formation by I pilus-determining plasmids in *Escherichia coli* K-12. J. Bacteriol. **125**:811–817.
55. **Davis, E. J., and J. Henry.** 1982. Conjugal transfer replication of R64*drd11* plasmid DNA in the donor cells of *Escherichia coli* K-12. Mol. Gen. Genet. **187**:305–309.
56. **Dougan, G., M. Saul, G. Warren, and D. Sherratt.** 1978. A functional map of plasmid ColE1. Mol. Gen. Genet. **158**:325–327.
57. **Dougan, G., and D. Sherratt.** 1977. The transposon Tn*1* as a probe for studying ColE1 structure and function. Mol. Gen. Genet. **151**:151–160.
58. **Ebina, Y., F. Kishi, T. Nakazawa, and A. Nakazawa.** 1979. Gene expression *in vitro* of colicin E1 plasmid. Nucleic Acids Res. **7**:639–649.
59. **Eisenberg, S., J. Griffith, and A. Kornberg.** 1977. φX174 *cistron A* protein is a multifunctional enzyme in DNA replication. Proc. Natl. Acad. Sci. U.S.A. **74**:3198–3202.
60. **Eisenberg, S., J. F. Scott, and A. Kornberg.** 1976. Enzymatic replication of viral and complementary strands of duplex DNA of phage φX174 proceeds by separate mechanisms. Proc. Natl. Acad. Sci. U.S.A. **73**:3151–3155.
61. **Everett, R., and N. Willetts.** 1980. Characterization of an *in vivo* system for nicking at the origin of conjugal DNA transfer of the sex factor F. J. Mol. Biol. **136**:129–150.
62. **Everett, R., and N. Willetts.** 1982. Cloning, mutation, and location of the F origin of conjugal transfer. EMBO J. **1**:747–753.
63. **Falkow, S., P. Guerry, R. W. Hedges, and N. Datta.** 1974. Polynucleotide sequence relationships among plasmids of the I compatibility complex. J. Gen. Microbiol. **85**:65–76.
64. **Falkow, S., L. S. Tompkins, R. P. Silver, P. Guerry, and D. J. LeBlanc.** 1971. The replication of R-factor DNA in *Escherichia coli* K-12 following conjugation. Ann. N.Y. Acad. Sci. **182**:153–171.
65. **Fenwick, R. G., Jr., and R. Curtiss III.** 1973. Conjugal deoxyri-

bonucleic acid replication by *Escherichia coli* K-12: stimulation in *dnaB*(Ts) donors by minicells. J. Bacteriol. **116**:1212–1223.
66. **Fenwick, R. G., Jr., and R. Curtiss III.** 1973. Conjugal deoxyribonucleic acid replication by *Escherichia coli* K-12: effect of chloramphenicol and rifampin. J. Bacteriol. **116**:1224–1235.
67. **Fenwick, R. G., Jr., and R. Curtiss III.** 1973. Conjugal deoxyribonucleic acid replication by *Escherichia coli* K-12: effect of nalidixic acid. J. Bacteriol. **116**:1236–1246.
68. **Finnegan, D., and N. Willetts.** 1973. The site of action of the F transfer inhibitor. Mol. Gen. Genet. **127**:307–316.
69. **Finnegan, J., and D. Sherratt.** 1982. Plasmid ColE1 conjugal mobility: the nature of *bom* a region required in *cis* for transfer. Mol. Gen. Genet. **185**:344–351.
70. **Fisher, K. W., and M. B. Fisher.** 1968. Nalidixic acid inhibition of DNA transfer in *Escherichia coli* K12. Cold Spring Harbor Symp. Quant. Biol. **33**:629–633.
71. **Folkhard, W., K. R. Leonard, S. Malsey, D. A. Marvin, J. Dubochet, A. Engel, M. Achtman, and R. Helmuth.** 1979. X-ray diffraction and electron microscope studies on the structure of bacterial F pili. J. Mol. Biol. **130**:145–160.
72. **Freifelder, D. R., and D. Freifelder.** 1968. Studies on *Escherichia coli* sex factors. I. Specific labeling of F'Lac DNA. J. Mol. Biol. **32**:15–23.
73. **Gaffney, D., R. Skurray, and N. Willetts.** 1983. Regulation of the F conjugation genes studied by hybridization and *tra-lacZ* fusion. J. Mol. Biol. **168**:103–122.
74. **Geider, K., E. Beck, and H. Schaller.** 1978. An RNA transcribed from DNA at the origin of phage fd single strand to replicative form conversion. Proc. Natl. Acad. Sci. U.S.A. **75**:645–649.
75. **Geider, K., and H. Hoffmann-Berling.** 1981. Proteins controlling the helical structure of DNA. Annu. Rev. Biochem. **50**:233–260.
76. **Geider, K., and A. Kornberg.** 1974. Conversion of the M13 viral single strand to the double-stranded replicative forms by purified proteins. J. Biol. Chem. **249**:3999–4005.
77. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. **50**:879–910.
78. **Gilbert, W., and D. Dressler.** 1968. DNA replication: the rolling circle model. Cold Spring Harbor Symp. Quant. Biol. **33**:473–484.
79. **Glassberg, J., R. R. Meyer, and A. Kornberg.** 1979. Mutant single-strand binding protein of *Escherichia coli*: genetic and physiological characterization. J. Bacteriol. **140**:14–19.
80. **Grindley, N. D. F., G. O. Humphreys, and E. S. Anderson.** 1973. Molecular studies of R factor compatibility groups. J. Bacteriol. **115**:387–398.
81. **Grinter, N.** 1981. Analysis of chromosome mobilization using hybrids between plasmid RP4 and a fragment of bacteriophage λ carrying IS1. Plasmid **5**:267–276.
82. **Guiney, D. G., and D. R. Helinski.** 1975. Relaxation complexes of plasmid DNA protein. III. Association of protein with the 5' terminus of the broken DNA strand in the relaxed complex of plasmid ColE1. J. Biol. Chem. **250**:8796–8803.
83. **Guiney, D. G., and D. R. Helinski.** 1979. The DNA-protein relaxation complex of plasmid RK2: location of the site-specific nick in the region of the proposed origin of transfer. Mol. Gen. Genet. **176**:183–189.
84. **Guiney, D. G., and E. Yakobson.** 1983. Location and nucleotide sequence of the transfer origin of the broad host range plasmid RK2. Proc. Natl. Acad. Sci. U.S.A. **80**:3595–3598.
85. **Guyer, M. S., and A. J. Clark.** 1976. *cis*-dominant, transfer-deficient mutants of the *Escherichia coli* K-12 F sex factor. J. Bacteriol. **125**:233–247.
86. **Guyer, M. S., N. Davidson, and A. J. Clark.** 1977. Heteroduplex analysis of *traΔ* F' plasmids and the mechanism of their formation. J. Bacteriol. **131**:970–980.
87. **Hane, M. W.** 1971. Some effects of nalidixic acid on conjugation in *Escherichia coli* K-12. J. Bacteriol. **105**:46–56.
88. **Harden, V., and E. Meynell.** 1972. Inhibition of gene transfer by antiserum and identification of serotypes of sex pili. J. Bacteriol. **109**:1067–1074.
89. **Hardy, K. G.** 1975. Colicinogeny and related phenomena. Bacteriol. Rev. **39**:464–515.
90. **Hiraga, S., and T. Saitoh.** 1975. F deoxyribonucleic acid transferred to recipient cells in the presence of rifampin. J. Bacteriol. **121**:1000–1006.
91. **Hollom, S., and R. H. Pritchard.** 1965. Effect of inhibition of DNA synthesis on mating in *Escherichia coli* K12. Genet. Res. **6**:479–483.
92. **Ihler, G., and W. D. Rupp.** 1969. Strand-specific transfer of donor DNA during conjugation in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **63**:138–143.
93. **Inselburg, J.** 1974. Replication of colicin E1 plasmid DNA in minicells from a unique replication initiation site. Proc. Natl. Acad. Sci. U.S.A. **71**:2256–2259.
94. **Inselburg, J.** 1977. Studies of colicin E1 plasmid functions by analysis of deletions and Tn*A* insertions of the plasmid. J. Bacteriol. **132**:332–340.
95. **Inselburg, J., and B. Applebaum.** 1978. Proteins synthesized in minicells containing plasmid ColE1 and its mutants. J. Bacteriol. **133**:1444–1451.
96. **Inselburg, J., and P. Ware.** 1979. A complementation analysis of mobilization deficient mutants of the plasmid ColE1. Mol. Gen. Genet. **172**:211–219.
97. **Johnson, D., R. Everett, and N. Willetts.** 1981. Cloning of F DNA fragments carrying the origin of transfer *oriT* and the fertility inhibition gene *finP*. J. Mol. Biol. **153**:187–202.
98. **Kennedy, N., L. Beutin, M. Achtman, R. Skurray, U. Rahmsdorf, and P. Herrlich.** 1977. Conjugation proteins encoded by the F sex factor. Nature (London) **270**:580–585.
99. **Kingsman, A., and N. Willetts.** 1978. The requirements for conjugal DNA synthesis in the donor strain during F *lac* transfer. J. Mol. Biol. **122**:287–300.
100. **Kline, B. C., and D. R. Helinski.** 1971. F_1 sex factor of *Escherichia coli*. Size and purification in the form of a strand-specific relaxation complex of supercoiled deoxyribonucleic acid and protein. Biochemistry **10**:4975–4980.
101. **Kolodkin, A. L., M. A. Capage, E. I. Golub, and K. B. Low.** 1983. F sex factor of *Escherichia coli* K-12 codes for a single-stranded DNA binding protein. Proc. Natl. Acad. Sci. U.S.A. **80**:4422–4426.
102. **Kornberg, A.** 1980. DNA replication. W. H. Freeman and Co., San Francisco.
103. **Kornberg, A.** 1982. 1982 Supplement to DNA replication. W. H. Freeman and Co., San Francisco.
104. **Kreuzer, K. N., and N. R. Cozzarelli.** 1979. *Escherichia coli* mutants thermosensitive for deoxyribonucleic acid gyrase subunit A: effects on deoxyribonucleic acid replication, transcription, and bacteriophage growth. J. Bacteriol. **140**:424–435.
105. **Kuhn, B., M. Abdel-Monem, H. Krell, and H. Hoffmann-Berling.** 1979. Evidence for two mechanisms for DNA unwinding catalyzed by DNA helicases. J. Biol. Chem. **254**:11343–11350.
106. **Kupersztoch-Portnoy, Y. M., M. A. Lovett, and D. R. Helinski.** 1974. Strand and site specificity of the relaxation event for the relaxation complex of the antibiotic resistance plasmid R6K. Biochemistry **13**:5484–5490.
107. **Kupersztoch-Portnoy, Y. M., G. L. G. Miklos, and D. R. Helinski.** 1974. Properties of the relaxation complexes of supercoiled deoxyribonucleic acid and protein of the R plasmids R64, R28K, and R6K. J. Bacteriol. **120**:545–548.
108. **Lanka, E., and P. T. Barth.** 1981. Plasmid RP4 specifies a deoxyribonucleic acid primase involved in its conjugal transfer and maintenance. J. Bacteriol. **148**:769–781.
109. **Lanka, E., E. Scherzinger, E. Günther, and H. Schuster.** 1979. A DNA primase specified by I-like plasmids. Proc. Natl. Acad. Sci. U.S.A. **76**:3632–3636.
110. **Lawn, A. M., and E. Meynell.** 1972. Antibody-stimulated increase in sex pili in R^+ enterobacteria. Nature (London) **235**:441–442.
111. **Lovett, M. A., D. G. Guiney, and D. R. Helinski.** 1974. Relaxation complexes of plasmids ColE1 and ColE2: unique site of the nick in the open circular DNA of the relaxed complexes. Proc. Natl. Acad. Sci. U.S.A. **71**:3854–3857.

112. **Lovett, M. A., and D. R. Helinski.** 1975. Relaxation complexes of plasmid DNA and protein. II. Characterization of the proteins associated with the unrelaxed and relaxed complexes of plasmid ColE1. J. Biol. Chem. **250**:8790–8795.
113. **Lovett, M. A., L. Katz, and D. R. Helinski.** 1974. Unidirectional replication of plasmid ColE1 DNA. Nature (London) **251**:337–340.
114. **Ludwig, R. A., and E. Johansen.** 1980. DnaG-suppressing variants of R68 · 45 with enhanced chromosome donating ability in *Rhizobium*. Plasmid **3**:359–361.
115. **Machida, Y., T. Okazaki, and R. Okazaki.** 1977. Discontinuous replication of replicative form DNA from bacteriophage ϕX174. Proc. Natl. Acad. Sci. U.S.A. **74**:2776–2779.
116. **Manning, P. A., and M. Achtman.** 1979. Cell-to-cell interactions in conjugating *Escherichia coli*: the involvement of the cell envelope, p. 409–447. *In* M. Inouye (ed.), Bacterial outer membranes: biogenesis and functions. John Wiley & Sons, Inc., New York.
117. **Manning, P. A., G. Morelli, and M. Achtman.** 1981. *traG* protein of the F sex factor of *Escherichia coli* K-12 and its role in conjugation. Proc. Natl. Acad. Sci. U.S.A. **78**:7487–7491.
118. **Marians, K. J., W. Soeller, and S. L. Zipursky.** 1982. Maximal limits of the *Escherichia coli* replication factor Y effector site sequences in pBR322 DNA. J. Biol. Chem. **257**:5656–5662.
119. **Marinus, M. G., and E. A. Adelburg.** 1970. Vegetative replication and transfer replication of deoxyribonucleic acid in temperature-sensitive mutants of *Escherichia coli* K-12. J. Bacteriol. **104**:1266–1272.
120. **Matsubara, K.** 1968. Properties of sex factor and related episomes isolated from purified *Escherichia coli* zygote cells. J. Mol. Biol. **38**:89–108.
121. **Matthes, M., and D. T. Denhardt.** 1980. The mechanism of replication of ϕX174 DNA. XVI. Evidence that the ϕX174 viral strand is synthesized discontinuously. J. Mol. Biol. **136**:45–63.
122. **Matthes, M., P. J. Weisbeek, and D. T. Denhardt.** 1982. Mechanism of replication of bacteriophage ϕX174. XIX. Initiation of ϕX174 viral strand DNA synthesis at internal sites on the genome. J. Virol. **42**:301–305.
123. **Maturin, L. J., Sr., and R. Curtiss III.** 1981. Role of ribonucleic acid synthesis in conjugational transfer of chromosomal and plasmid deoxyribonucleic acids. J. Bacteriol. **146**:552–563.
124. **Morris, C. F., C. L. Hershberger, and R. Rownd.** 1973. Strand-specific nick in open circular R-factor deoxyribonucleic acid: attachment of the linear strand to a proteinaceous cellular component. J. Bacteriol. **114**:300–308.
125. **Nomura, N., R. L. Low, and D. S. Ray.** 1982. Identification of ColE1 DNA sequences that direct single strand-to-double strand conversion by a ϕX174 type mechanism. Proc. Natl. Acad. Sci. U.S.A. **79**:3153–3157.
126. **Nordheim, A.** 1979. Charakterizierung von bacteriellen Plasmid DNA-Protein Relaxationskomplexen. Ph.D. thesis, Freie Universität. Berlin.
127. **Nordheim, A., T. Hashimoto-Gotoh, and K. T. Timmis.** 1980. Location of two relaxation nick sites in R6K and single sites in pSC101 and RSF1010 close to origins of vegetative replication: implication for conjugal transfer of plasmid deoxyribonucleic acid. J. Bacteriol. **144**:923–932.
128. **Novotny, C., E. Raizen, W. S. Knight, and C. C. Brinton, Jr.** 1969. Functions of F pili in mating-pair formation and male bacteriophage infection studied by blending spectra and reappearance kinetics. J. Bacteriol. **98**:1307–1319.
129. **Ogawa, T., and T. Okazaki.** 1980. Discontinuous DNA replication. Annu. Rev. Biochem. **49**:421–457.
130. **Ohki, M., and J. Tomizawa.** 1968. Asymmetric transfer of DNA strands in bacterial conjugation. Cold Spring Harbor Symp. Quant. Biol. **33**:651–657.
131. **Ohmori, H., and J. Tomizawa.** 1979. Nucleotide sequence of the region required for maintenance of colicin E1 plasmid. Mol. Gen. Genet. **176**:161–170.
132. **Oka, A., N. Nomura, M. Morita, H. Sugisaki, K. Sugimoto, and M. Takanami.** 1979. Nucleotide sequence of small ColE1 derivatives: structure of the regions essential for autonomous replication and colicin E1 immunity. Mol. Gen. Genet. **172**:151–159.
133. **Orr, E., N. F. Fairweather, I. B. Holland, and R. H. Pritchard.** 1979. Isolation and characterization of a strain carrying a conditional lethal mutation in the *cou* gene of *Escherichia coli* K12. Mol. Gen. Genet. **177**:103–112.
134. **Ou, J. T.** 1975. Mating signal and DNA penetration deficiency in conjugation between male *Escherichia coli* and minicells. Proc. Natl. Acad. Sci. U.S.A. **72**:3721–3725.
135. **Ou, J. T., and T. F. Anderson.** 1970. Role of pili in conjugation. J. Bacteriol. **102**:648–654.
136. **Paranchych, W.** 1975. Attachment, ejection and penetration stages of the RNA phage infectious process, p. 85–111. *In* N. D. Zinder (ed.), RNA phages. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
137. **Queen, C., and M. Rosenberg.** 1981. A promoter of pBR322 activated by cAMP receptor protein. Nucleic Acids Res. **9**:3365–3377.
138. **Reeves, P., and N. Willetts.** 1974. Plasmid specificity of the origin of transfer of sex factor F. J. Bacteriol. **120**:125–130.
139. **Rowen, L., and A. Kornberg.** 1978. Primase, the *dnaG* protein of *Escherichia coli*. J. Biol. Chem. **253**:758–764.
140. **Rowen, L., and A. Kornberg.** 1978. A ribo-deoxyribonucleotide primer synthesized by primase. J. Biol. Chem. **253**:770–774.
141. **Rupp, W. D., and G. Ihler.** 1968. Strand selection during bacterial mating. Cold Spring Harbor Symp. Quant. Biol. **33**:647–650.
142. **Sarathy, P. V., and O. Siddiqi.** 1973. DNA synthesis during bacterial conjugation. II. Is DNA replication in the Hfr obligatory for chromosome transfer? J. Mol. Biol. **78**:443–451.
143. **Sasakawa, C., and M. Yoshikawa.** 1978. Requirements for suppression of a *dnaG* mutation by an I-type plasmid. J. Bacteriol. **133**:485–491.
144. **Shlomai, J., and A. Kornberg.** 1980. An *Escherichia coli* replication protein that recognizes a unique sequence within a hairpin region in ϕX174 DNA. Proc. Natl. Acad. Sci. U.S.A. **77**:799–803.
145. **Soberon, X., L. Covarrubias, and F. Bolivar.** 1980. Construction and characterization of new cloning vehicles. IV. Deletion derivatives of pBR322 and pBR325. Gene **9**:287–305.
146. **Soeller, W. C., and K. J. Marians.** 1982. Deletion mutants defining the *Escherichia coli* replication factor Y effector site sequences in pBR322 DNA. Proc. Natl. Acad. Sci. U.S.A. **79**:7253–7257.
147. **Spanos, A., S. G. Sedgwick, G. T. Yarranton, U. Hübscher, and G. R. Banks.** 1981. Detection of the catalytic activities of DNA polymerases and their associated exonucleases following SDS-polyacrylamide gel electrophoresis. Nucleic Acids Res. **9**:1825–1839.
148. **Staudenbauer, W. L.** 1978. Structure and replication of the colicin E1 plasmid. Curr. Top. Microbiol. Immunol. **83**:93–156.
149. **Stuitze, A. R., C. E. Spelt, E. Veltkamp, and H. J. J. Nijkamp.** 1981. Identification of mutations affecting replication control of plasmid CloDF13. Nature (London) **290**:264–267.
150. **Sugino, A., C. L. Peebles, K. N. Kreuzer, and N. Cozzarelli.** 1977. Mechanism of action of nalidixic acid: purification of *Escherichia coli nalA* gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. Proc. Natl. Acad. Sci. U.S.A. **74**:4767–4771.
151. **Sugino, Y., J. Tomizawa, and T. Kakefuda.** 1975. Location of non-DNA components of closed circular colicin E1 plasmid DNA. Nature (London) **253**:652–654.
152. **Sumida-Yasumoto, C., J.-E. Ikeda, E. Benz, K. J. Marians, R. Vicuna, S. Sugrue, S. L. Zipursky, and J. Hurwitz.** 1978. Replication of ϕX174 DNA: in vitro synthesis of ϕX RFI DNA and circular, single-stranded DNA. Cold Spring Harbor Symp. Quant. Biol. **43**:311–329.
153. **Sutcliffe, J. G.** 1978. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
154. **Thompson, R., and M. Achtman.** 1978. The control region of

the F sex factor DNA transfers cistrons: restriction mapping and DNA cloning. Mol. Gen. Genet. **165**:295–304.

155. **Thompson, R., and M. Achtman.** 1979. Nucleotide sequence of the F DNA transfer origin, p. 331–338. *In* S. W. Glover and L. O. Butler (ed.), Proceedings, 4th European Meeting on Bacterial Transformation and Transfection. Cotswold Press Ltd., Oxford.
156. **Thompson, R., and L. Taylor.** 1982. Promoter mapping and DNA sequencing of the F plasmid transfer genes *traM* and *traJ*. Mol. Gen. Genet. **188**:513–518.
157. **Tomizawa, J.** 1978. Replication of colicin E1 plasmid DNA *in vitro*, p. 797–826. *In* I. Molineux and M. Kohiyama (ed.), DNA synthesis—present and future. Plenum Press, New York.
158. **Tomizawa, J., H. Ohmori, and R. E. Bird.** 1977. Origin of replication of colicin E1 plasmid DNA. Proc. Natl. Acad. Sci. U.S.A. **74**:1865–1869.
159. **Tomizawa, J., Y. Sakakibara, and T. Kakefuda.** 1974. Replication of colicin E1 plasmid DNA in cell extracts. Origin and direction of replication. Proc. Natl. Acad. Sci. U.S.A. **71**:2260–2264.
160. **Twigg, A. J., and D. Sherratt.** 1980. Trans-complementable copy-number mutants of plasmid ColE1. Nature (London) **283**:216–218.
161. **Van de Pol, H., E. Veltkamp, and H. J. J. Nijkamp.** 1978. Genetic analysis of the mobilization of the non-conjugative plasmid CloDF13. Mol. Gen. Genet. **160**:139–149.
162. **Van der Ende, A., R. Teertstra, H. G. A. M. van der Avoort, and P. J. Weisbeek.** 1983. Initiation signals for complementary strand DNA synthesis on single-stranded plasmid DNA. Nucleic Acids Res. **11**:4957–4975.
163. **Van der Ende, A., R. Teertstra, and P. J. Weisbeek.** 1983. n′ protein activator sites of plasmid pBR322 are not essential for its DNA replication. J. Mol. Biol. **167**:751–756.
164. **Vapnek, D., M. B. Lipman, and W. D. Rupp.** 1971. Physical properties and mechanism of transfer of R factors in *Escherichia coli*. J. Bacteriol. **108**:508–514.
165. **Vapnek, D., and W. D. Rupp.** 1970. Asymmetric segregation of the complementary sex-factor DNA strands during conjugation in *Escherichia coli*. J. Mol. Biol. **53**:287–303.
166. **Vapnek, D., and W. D. Rupp.** 1971. Identification of individual sex-factor DNA strands and their replication during conjugation in thermosensitive DNA mutants of *Escherichia coli*. J. Mol. Biol. **60**:413–424.
167. **Wang, P. Y., and V. N. Iyer.** 1977. Suppression and enhancement of temperature sensitivity of *dnaB* mutation of *Escherichia coli* K12 by conjugative plasmids. Plasmid **1**:19–33.
168. **Wang, P. Y., and V. N. Iyer.** 1978. Analogs of the *dnaB* gene of *Escherichia coli* K-12 associated with conjugative R plasmids. J. Bacteriol. **134**:765–770.
169. **Warren, G. J., and A. J. Clark.** 1980. Sequence-specific recombination of plasmid ColE1. Proc. Natl. Acad. Sci. U.S.A. **77**:6724–6728.
170. **Warren, G. J., M. W. Saul, and D. J. Sherratt.** 1979. ColE1 plasmid mobility: essential and conditional functions. Mol. Gen. Genet. **170**:103–107.
171. **Warren, G., and D. Sherratt.** 1977. Complementation of transfer deficient ColE1 mutants. Mol. Gen. Genet. **151**:197–201.
172. **Warren, G. J., A. J. Twigg, and D. J. Sherratt.** 1978. ColE1 plasmid mobility and relaxation complex. Nature (London) **274**:259–261.
173. **Watson, J., L. Schmidt, and N. Willetts.** 1980. Cloning the TraI region of RP1. Plasmid **4**:175–183.
174. **Weiner, J. H., L. L. Bertsch, and A. Kornberg.** 1975. The deoxyribonucleic acid unwinding protein of *Escherichia coli*. J. Biol. Chem. **250**:1972–1980.
175. **Wickner, S.** 1977. DNA or RNA priming of bacteriophage G4 DNA synthesis of *Escherichia coli dnaG* protein. Proc. Natl. Acad. Sci. U.S.A. **74**:2815–2819.
176. **Wilkins, B. M.** 1975. Partial suppression of the phenotype of *Escherichia coli* K-12 *dnaG* mutants by some I-like conjugative plasmids. J. Bacteriol. **122**:899–904.
177. **Wilkins, B. M., G. J. Boulnois, and E. Lanka.** 1981. A plasmid DNA primase active in discontinuous bacterial DNA replication. Nature (London) **290**:217–221.
178. **Wilkins, B. M., and S. E. Hollom.** 1974. Conjugational synthesis of F *lac*$^+$ and ColI DNA in the presence of rifampicin and in *Escherichia coli* K12 mutants defective in DNA synthesis. Mol. Gen. Genet. **134**:143–156.
179. **Willetts, N.** 1970. The interaction of an I-like R factor and transfer-deficient mutants of F *lac* in *E. coli* K12. Mol. Gen. Genet. **108**:365–373.
180. **Willetts, N.** 1972. Location of the origin of transfer of the sex factor F. J. Bacteriol. **112**:773–778.
181. **Willetts, N.** 1977. The transcriptional control of fertility in F-like plasmids. J. Mol. Biol. **112**:141–148.
182. **Willetts, N.** 1980. Interactions between the F conjugal transfer system and CloDF13::TnA plasmids. Mol. Gen. Genet. **180**:213–217.
183. **Willetts, N.** 1981. Sites and systems for conjugal DNA transfer in bacteria, p. 207–215. *In* S. B. Levy, R. C. Clowes, and E. L. Koenig (ed.), Molecular biology, pathogenicity, and ecology of bacterial plasmids. Plenum Press, New York.
184. **Willetts, N., and C. Crowther.** 1981. Mobilization of the nonconjugative IncQ plasmid RSF1010. Genet. Res. **37**:311–316.
185. **Willetts, N., and J. Maule.** 1979. Investigations of the F conjugation gene *traI*: *traI* mutants and λ*traI* transducing phages. Mol. Gen. Genet. **169**:325–336.
186. **Willetts, N., and R. Skurray.** 1980. The conjugative system of F-like plasmids. Annu. Rev. Genet. **14**:41–76.
187. **Zipursky, S. L., and K. J. Marians.** 1980. Identification of two *Escherichia coli* factor Y effector sites near the origins of replication of the plasmids ColE1 and pBR322. Proc. Natl. Acad. Sci. U.S.A. **77**:6521–6525.
188. **Zipursky, S. L., and K. J. Marians.** 1981. *Escherichia coli* factor Y sites of plasmid pBR322 can function as origins of DNA replication. Proc. Natl. Acad. Sci. U.S.A. **78**:6111–6115.